

The Journal of Parasitology

Volume 23

AUGUST, 1937

Number 4

INTESTINAL PROTOZOA FROM GALAPAGOS TORTOISES (WITH DESCRIPTIONS OF THREE NEW SPECIES)

QUENTIN M. GEIMAN AND RALPH WICHTERMAN

Department of Comparative Pathology, Harvard Medical School, Zoology
Laboratory, University of Pennsylvania and Biology
Department, Temple University

INTRODUCTION

A large Galapagos tortoise, *Testudo hoodensis*, in the collection of the Zoological Gardens at Philadelphia died during the night of September 17-18, 1933. The specimen was autopsied at the Laboratory of Comparative Pathology (Philadelphia) on the following day. The cause of death was not determined, but it was believed that the one to three pounds of large pebbles and a putrified mass of leaves and débris in the greatly distended and decomposing stomach and intestine were partly responsible.¹ A microscopic examination of the intestinal contents (by Q. M. G.) revealed three species of Protozoa belonging to the genera *Balantidium*, *Nyctotherus*, and *Trichomonas*. The *Nyctotherus* sp. appeared to be morphologically different from previously described species of this genus and later proved to be a new species. Consequently feces from the four remaining "Galapagos" tortoises in the collection, (*Testudo hoodensis* from Hood Island, *T. elephantina* from Aldabra Island in the Indian Ocean, and two *T. vicina* from an unknown source) were examined for Protozoa at varying intervals over a period of approximately a year and a half. This prolonged study of freshly collected feces and stained preparations revealed two additional organisms besides those found at the autopsy of *T. hoodensis*, a larger distinct *Nyctotherus* sp. and an *Endamoeba* sp. Thus we wish to describe *Nyctotherus kyphodes* n. sp., *Nyctotherus teleacus* n. sp., *Endamoeba insolita* n. sp. and a *Trichomonas* sp. Some notes on *Balantidium testudinis* are also included.

This investigation was carried on at the Laboratory of Comparative Pathology, Philadelphia Zoological Gardens, Zoological Laboratory of

¹ Benedict (1932) mentions the passage of varying amounts of pebbles in the feces of two South American Gopher tortoises, *Testudo denticulata*. However, he does not give autopsy records when the animals were sacrificed.

the University of Pennsylvania, Marine Biological Laboratory, Woods Hole, Massachusetts, Department of Comparative Pathology, Harvard Medical School and Temple University. We wish to express our indebtedness to Dr. D. H. Wenrich for valuable advice given throughout the work, and to Dr. E. E. Tyzzer for suggestions in the final stages of the study. We extend our sincere appreciation also to Drs. Fox and H. L. Ratcliffe of the Laboratory of Comparative Pathology, Zoological Gardens of Philadelphia, for their permission and splendid cooperation that made the material available to us for study.

MATERIAL AND METHODS

Three of the giant Galapagos tortoises *Testudo elephantina* and two *T. vicina* were placed on exhibition at the Zoological Gardens of Philadelphia approximately eight years ago. The two specimens of *T. hoodensis* were presented to the collection by Gifford Pinchot. In the winter, the tortoises are kept in two cages in the Reptile House. During the warm summer months they occupy one house for shelter and an adjoining cage in the open air. This caging arrangement made it possible to obtain fresh fecal material whenever available throughout the year.

Observations and drawings were made from both living and fixed protozoons. The living protozoons were studied on smears of feces to which was added 0.65 per cent physiological salt solution. Preparations, made by ringing the coverslip with melted vaseline, were used for prolonged study during periods up to two weeks. Occasionally, intestinal contents containing the parasites were kept in small shallow dishes and then diluted with Ringer's solution. Saline concentrations of 0.5 per cent to 0.8 per cent appeared to be satisfactory.

The fixatives employed in the study were Schaudinn's fluid plus 5 per cent of glacial acetic acid, used at room temperature and at 45° C., Bouin's-urea modification of Grassé (1926) and Bouin's fluid.

The stains used and giving uniformly good results were Mayer's haemalum counterstained with alcoholic eosin and Heidenhain's iron-alum haematoxylin counterstained in some instances with Orange "G." In using Mayer's haemalum for the large ciliates, better nuclear detail was secured by placing the stained and washed smear preparations into a petri dish of distilled water to which was added a few drops of ammonium hydroxide. Smears were left here for a minute, then dehydrated, counterstained, cleared and mounted. The standard method of staining with Heidenhain's iron-alum haematoxylin was used for the amoeba and the flagellate, but better results were obtained with the ciliates by destaining with a saturated aqueous solution of picric acid. (Tuan, 1930.)

OBSERVATIONS

Nyctotherus kyphodes n. sp.

A. Trophozoite

(a) *Shape and size.* When viewed dorsally, this species is broadly oval in shape with a tail-like posterior prolongation and though somewhat flattened, the posterior end is thicker than the anterior end (Plate I, fig. 1). Seen from the edge, the dorsal surface slopes gradually down to the anterior end of the animal. While the ventral surface is generally flattened or slightly convex, the dorsal surface, on the other hand, is more convex (Fig. 4). On the side of the cytopharynx is a groove running nearly the entire length of the body (Figs. 1 and 4). Opposite the cytopharynx, when viewed dorsally, there is seen a border or flange roughly similar to that described for *N. cheni* (Wichterman, 1934). This flange, however, loses its identity as it reaches the posterior part of the animal (Fig. 1). Measurements of 60 individuals showed the average length to be $191\ \mu$ (range $135\ \mu$ to $245\ \mu$) and the average width $108\ \mu$ (range $65\ \mu$ to $150\ \mu$). The organism moves about gracefully in the wet preparations and due to its shape, it easily burrows under debris. In the body, especially anterior to the large macronucleus, may occasionally be observed ellipsoidal and corpuscle-like paraglycogen bodies which stain intensely with iodine.

(b) *Pellicle, peripheral cilia and peristomal apparatus.* The peristomal groove begins near the apex of the protozoon and extends about one-third the animal's entire length to meet the cytopharynx. The cytopharynx extends obliquely inward with little or no bending except at its extreme end where there is a sharp bend. Coming from the end of the cytopharynx is the continuation of the subpharyngeal canal (Higgins, 1929) which curves around toward the cytostome. The posterior end of the body, dorsally, is characterized by an extension in the manner indicated on plate I, fig. 1. Many closely-set ciliary lines run parallel to the longitudinal axis of the body. From them emanate the cilia which appear to be of moderate length (approximately $8\ \mu$). The cilia beat with a metasynchronous action except for those at the posterior tip of the body. Here the cilia are stiff and beat with a quick vibratile motion.

The peristomal groove and cytopharynx have closely set membranelles which are fine in structure.

The oral bristle, discovered by Stein (1859-78) was observed in this species only on a few occasions.

(c) *Contractile vacuole and special structures.* One very large contractile vacuole is found in the posterior region of the body usually located nearer the cytostomal side of the body. Intimately associated with the contractile vacuole is a deeply invaginated chamber which is lined with

long, fine cilia (Text fig. 1). This ciliated chamber measures in large individuals $40\ \mu$ in length and varies considerably in width. In young forms it appears to be present but is not conspicuous, due usually to the densely packed paraglycogen bodies present. The anterior part of the chamber usually tapers into a point where it meets the contractile vacuole. At times the chamber appears to be forked at the anterior end, one branch going to the contractile vacuole while the other at a slightly lower level, seems to end blindly in the cytoplasm. The chamber is widest at the point where it emerges from the dorsal surface of the body, to form a slit or cleft through the pellicle. This ciliated chamber reminds one of a cloaca found in metazoan animals. The contractile vacuole was observed to take slightly more than a minute to expand and three to four seconds to empty. It empties into the anterior part of the chamber, the connection of vacuole to chamber being most conspicuous at the time of excretion. Besides excretion, egestion also takes place through this structure. Also near the anterior part of the chamber there is an active churning of the protoplasm, and small bodies about to be egested are pushed into the chamber. Once in the chamber, the particles spin about due to the actively beating cilia within, then finally pass out the posterior opening of the chamber. The two physiological processes of excretion and egestion were observed going on simultaneously on many occasions.

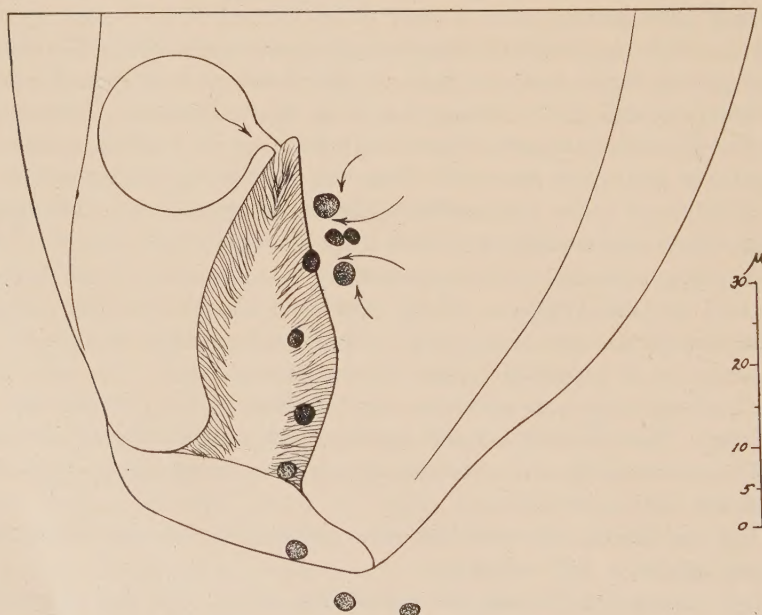


FIG. 1. A camera lucida drawing of the posterior region of *N. kyphodes* as observed alive under the oil immersion lens. The expanded contractile vacuole is about to empty into the "cloaca-like" ciliated chamber while egestion is taking place at another part of the chamber. The arrows near the spherical particles represent actively moving endoplasm. The animal was $193\ \mu$ long.

(d) *Nuclei*. The macronucleus of *N. kyphodes* is exceedingly large, measuring $75\ \mu$ in length by $18\ \mu$ in width. It is roughly banana- or sausage-shaped and extends parallel to the cytopharynx (Fig. 1). Caryophore strands connect the nucleus (Grassé, 1926) with the ectosarc (Figs. 1 and 4). Seen in living and stained individuals, the macronucleus is clearly granular.

The micronucleus is found at the middle of the inner part of the macronucleus and lying close to or slightly under it. Studied alive under the oil immersion lens, it is apparent as a clear homogeneous body, oval in shape and measuring approximately $6.5\ \mu$ by $4\ \mu$.

B. Cysts

Throughout the study, many cysts were observed. Cysts of this species (Fig. 2) are for the most part broadly oval, and of 60 fresh ones measured, the average length was $160\ \mu$ (range $128\ \mu$ and $205\ \mu$) and the average width $93\ \mu$ (range $68\ \mu$ and $123\ \mu$). The cyst wall is slightly over $2\ \mu$ thick. Located near the end of the cyst but to one side or slightly eccentric is a thickening of the cyst wall in the form of a cap, which reminds one of the operculum described by Lucas (1928) for the cyst of *N. ovalis* from the cockroach. The color of the cyst is a light brown. Pellicular folds of the organism can be noted in some cysts together with clear ellipsoidal paraglycogen bodies in the center. Early differentiation of membranelles was observed on an actively moving organism in a fresh cyst (Fig. 3). The cysts are strongly resistant to fixation and staining. There is, too, a general resemblance of the cysts to worm eggs also present in the intestinal contents.

Nyctotherus teleacus n. sp.

A. Trophozoites

(a) *Shape and size*. This species of *Nyctotherus* is roughly plano-convex, the ventral surface being approximately flat while the dorsal surface is slightly convex or arched (Fig. 5). Viewed dorso-ventrally, the anterior end is rounded while the posterior end is pointed and somewhat abruptly turned toward the side opposite the cytopharynx. Most of the organisms are relatively slender but robust individuals were noted time and again. Measurements of 60 organisms gave an average length of $112\ \mu$ (range $90\ \mu$ to $143\ \mu$) and average width of $55\ \mu$ (range $43\ \mu$ to $70\ \mu$).

(b) *Pellicle, peripheral cilia and peristomal apparatus*. The pellicle on *N. teleaco* is quite thin and flexible. The animal can squeeze its body through extremely small spaces or openings. Running parallel to the longitudinal axis of the body are the ciliary lines which cover the general body surface. These lines converge however at the anterior and pos-

terior ends of the animal. In several, 26 to 28 lines were counted on the dorsal surface. Coming from these ciliary lines are the long cilia. The cilia are slightly longer at the anterior and posterior ends than at the sides. They are fewer in number within the arc formed at the posterior tip. At the extreme posterior tip are cilia which do not beat metasynchronously as do the others. Here the cilia as in *N. kyphodes*, radiate out from this end in a stiff, rigid manner and possess a quick vibratile motion.

The peristomal groove begins near the apex and extends nearly one-half way down the entire length of the animal where it approaches the cytopharynx. The cytopharynx extends obliquely inwards with little bending except at the inner end. Here there is a sharp bend posteriad and ventrad. Coming from this bend is the continuation of the subpharyngeal canal which curves around and points toward the cytostome.

The peristomal groove and cytopharynx have strong membranelles which are very active and flexible. The membranelles are longer in one peristomal groove than in the cytopharynx and longest where the groove meets the cytopharynx.

The oral bristle (Fig. 5) was observed several times in the study but the structure was not seen on all forms examined. It appears to lie on the floor of the cytopharynx and extends inwards for some distance where it is attached. It is usually much longer than the membranelles of the peristomal region and is distinct from them. The bristle does not take a part in the metasynchronous movement of the membranelles.

(c) *Contractile vacuole and special structures.* A single, fairly large contractile vacuole was present in the majority of forms observed. The vacuole is located between the cytopharynx and the posterior end of the body. It remains expanded for many minutes and was seen to empty on several occasions in 45 seconds. In some individuals, two contractile vacuoles were observed close together.

At the posterior dorsal part of the animal and situated below the contractile vacuole is a structure closely resembling that found in *N. kyphodes* and likewise directly concerned with excretion and egestion. It is a ciliated chamber, roughly cone-shaped with the widest part being most posterior and from which extend cilia set rather closely together. The anterior end of the structure dips ventrally and leads to the contractile vacuole. Egestion likewise takes place here as in the previously described species. Characteristic particles which were ingested were seen to be egested inside of four minutes while other particles were retained in the body for a longer period.

(d) *Nuclei.* The triangular macronucleus (Fig. 5) averaged $32\ \mu$ by $20\ \mu$ when 60 were measured. The longest side of the triangle lies directly on and parallel to the cytopharynx. Where the peristomal

groove meets the cytopharynx, the end of the macronucleus is bent up toward the anterior part of the animal. Directly opposite from this bent corner of the macronucleus is the caryophore.

The micronucleus which can be easily seen under the low power of the microscope, is usually broadly oval in shape. Average measurements give the size as $9\ \mu$ by $6\ \mu$. It is a clear, homogeneous, non-granular body which in the living condition is highly refractile with a greenish tint. In the prepared slides, this body stains more faintly than the macronucleus. It is invariably located on the side of the macronucleus which is nearest to the cytopharynx.

B. Cysts

The cyst of this species is elongately oval (Fig. 6). Measurements of 60 fresh cysts show the average length to be $90\ \mu$ (range $73\ \mu$ to $105\ \mu$) and the average width $52\ \mu$ (range $45\ \mu$ to $63\ \mu$). At one end of the cyst is found the operculum. Many empty cysts were noted with this operculum missing, indicating that excystation had already taken place. The cyst wall is approximately $1\ \mu$ to $2\ \mu$ thick, quite rigid and of a yellow color. In many cysts the protozoan could be observed with its somewhat folded pellicle and curved but faint ciliary lines (Fig. 7). Figure 7 is a cyst (*N. teleacus*) with the organism inside getting ready to excyst. In some cysts the macronucleus and micronucleus are quite distinct, the former being roughly spherical and granular and the latter oval and homogeneous. A vacuole, perhaps the contractile one, appears in most cysts being located usually near the operculum. Small ellipsoidal paraglycogen bodies measuring up to $4\ \mu$ in length were observed many times in the cysts, the bodies mainly concentrated in the center of it.

Balantidium testudinis Chagas, 1911

The *Balantidium*, which was obtained from at least three of the tortoises, is believed to be *Balantidium testudinis* Chagas, 1911. When Chagas described this species from *Testudo graeca*, no measurements were given and the cysts were not mentioned. Wenyon (1926) indicates that he saw this species in *T. radiata* and *T. calcarata*, but Fantham (1932) found and redescribed the species from three tortoises, identified as *Testudo angulata*. In the latter paper a brief general description of structures in the trophozoites and cysts was given, accompanied by measurements. The following notes are added to the description given by Chagas and Fantham.

A. Trophozoites

As seen in fresh preparations of feces, these ciliates were active, very voracious and in the majority of instances numerous. The shape (Fig. 8), corresponds to that given by Chagas and Fantham, but the size is

greater than that given by Fantham. Measurements of 50 individuals taken at random gave an average length of $73.3\ \mu$ (range $123.8\ \mu$ to $47.5\ \mu$) and average width of $52.3\ \mu$ (range $91.2\ \mu$ to $32.5\ \mu$).

Balantidia were successfully cultured (by R. W.) in abundant numbers. Cultivation was carried out in test tubes containing 10 cc. of sterilized Ringer's solution $R=2$ (modification of Drbohlav, 1925). Loeffler's dehydrated blood serum was added to make a 0.1 per cent solution followed by the addition of 1/100 of a gram of rice starch. A small amount of the fecal material containing balantidia was then placed in the above medium and the animals were cultured at room temperature.

The anterior cytostome and peristomal cilia are similar to those figured by Chagas, but there are also stout supporting fibrils around the cytostome and cytoplasm (Fig. 8). The contractile vacuoles and cytophyge were described by Fantham.

Since the shape and size of the macronucleus along with characteristics of shape and size of body have been shown by Hegner (1934) to be of diagnostic value in differentiating species of *Balantidium*, the outline of 20 macronuclei chosen at random are shown in text figure 2. The manner in which they vary is evident. The small micronucleus usually occupies a space at one side and near the middle of the macronucleus. Spherical macronuclei seen on a few occasions were believed to be evidence that the individuals containing them were exconjugants.

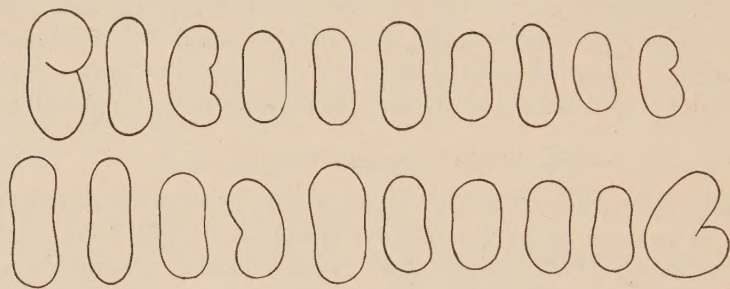


FIG. 2. Variation in size and shape of macronuclei in fixed and stained *Balantidium testudinis*. The twenty macronuclei were drawn at random with the aid of a camera lucida and are magnified approximately $\times 450$ (reduced $\frac{1}{3}$ for printing).

B. Cysts

The cysts are spherical and covered with a heavy cyst wall about $2\ \mu$ in thickness (Fig. 9). The range of size observed in 25 living cysts was $40.6\ \mu$ to $75.1\ \mu$ with an average diameter of $50.2\ \mu$. Very little of their internal structure could be observed, but in stained specimens (Fig. 9) the macronucleus appears to have maintained the normal shape, although smaller in size.

Endamoeba insolita n. sp.

A. Trophozoite

Active and cystic stages of this new species of endozoic amoeba were found on numerous occasions in feces from all turtles except *Testudo hoodensis*. The trophozoites are very sluggish even in freshly collected feces. If the organisms are watched carefully for a period of time, an occasional clear, narrow ectoplasmic pseudopodium can be observed along with the typical amoeboid flow of endoplasm containing the nucleus and food particles. In fixed and stained specimens (Plate II, figs. 10 and 12), the cytoplasm does not appear to be very dense. Ingested food particles (Fig. 11) are of the nature of vegetable debris and bacteria. When 75 fixed and stained organisms were measured, the average length was $21.1\ \mu$ (range $32\ \mu$ to $12.8\ \mu$), and the average width was $16.8\ \mu$ (range $23\ \mu$ to $12\ \mu$).

The vesicular nucleus (Fig. 10) contains a fairly large karyosome which is eccentric in the majority of cases. In approximately 25 per cent of the nuclei, the karyosome is concentric. A faint halo and spoke radii (Figs. 11, 13, and 16) are visible in some organisms. The peripheral chromatin is granular and unevenly placed under the nuclear membrane in fixed specimens from freshly collected feces (Figs. 10 and 12) but greater variation is found in nuclei of amoebae in older feces. The variation of nuclear structure brought about by degenerative changes is shown in plate II, fig. 14. No dividing nuclei of trophozoites were found on stained preparations although a small number of binucleate trophozoites were seen (Fig. 13). The range of size of the nuclear diameter in 75 trophozoites was $3.6\ \mu$ to $7\ \mu$ with an average of $5.2\ \mu$. The ratio of nuclear diameter to average diameter of trophozoites is .27.

Numerous attempts were made to cultivate the organism. The methods used by Ratcliffe and Geiman (1934) and Geiman and Ratcliffe (1936) and many variations were tried but were unsuccessful. The presence in the feces of *Blastocystis* sp., *Trichomonas* sp. and countless coprozoic protozoan cysts which excysted so readily in culture media, soon caused overgrowth that defeated successful cultivation of *Endamoeba insolita*.

B. Cysts

The cysts are formed by a process that is practically similar to that of other members of the genus. Trophozoites decrease in size to form precystic stages (Figs. 15 and 16). The unusual morphological characteristic of this species then manifests itself as a thick irregular gelatinous, brownish, membranous matrix begins to envelope the organism (Fig. 17). Debris and vegetable particles frequently adhere to the sticky cyst wall. When the cysts are fixed and stained, the irregularity is accentuated.

ated (Figs. 18 and 19) because of slight shrinkage. Occasionally evidence of a double cyst wall (Fig. 20) could be detected. Chromatoids are formed as the cyst wall is completed. They may be rod-like, ovoid, or irregular in shape (Figs. 18, 19 and 22). A glycogen vacuole is present in the majority of immature cysts.

Four nuclei are produced by division (Figs. 20 and 23) from the uninucleate primary stage of cystic development. The nuclei of the two (Figs. 21 and 22) and four (Figs. 24 and 25) nucleate stages become smaller but maintain to a large degree the normal structure seen in the nuclei of trophozoites. Cysts with eight nuclei were observed on only two occasions.

The size of the 50 stained cysts from two hosts varied between 12.8 μ and 19.5 μ with an average diameter of 15.7 μ .

Trichomonas sp.

Whenever a small flagellate, *Trichomonas* sp., was present in the feces, it occurred in large numbers. The flagellate is very actively motile, and the anterior end shows metabolic form changes. The body is spindle-shaped with posterior protrusions of an axostyle (Fig. 26). The average length of the organism was 9.8 μ (range 6.4 μ to 19 μ) and the average width was 5.8 μ (range 3.6 μ to 11 μ).

Three anterior flagella, approximately one and one-third times the length of the body arise from an anterior blepharoplast. The undulating membrane, which is attached only to the anterior half of the body gives rise to a trailing flagellum. No chromatic basal rod could be seen. The axostyle is characteristically hyaline in appearance and arises at the blepharoplast. Ingested food particles taken in at the mouth include vegetable debris and various bacteria which are large enough occasionally to cause body distortion.

The small vesicular nucleus is poor in chromatin with the exception of the small karyosome which stains deeply (Fig. 26). The karyosome may be centrally or peripherally placed. Dividing forms with nuclei in metaphase and telophase are shown in figures 27 and 28.

DISCUSSION

Van Denburgh (1914) says, "In recent and historic times gigantic land tortoises have existed only in certain isolated groups of islands in the Indian and Pacific Oceans, where the early explorers found them in almost incredible numbers." Their generations are supposed to have survived the Age of Giant Reptiles (Ditmars, 1907) and were widely distributed over the earth (Van Denburgh, 1914). Numerous small species of the genus *Testudo* are found in various parts of the world today, but many giant species are practically extinct due to the manner by which

the islands of the Indian and Pacific Oceans have been depleted of these reptiles by depredations of various landing parties over a period of at least three hundred years.

The giant tortoises are strictly terrestrial animals and a number of the species may weigh as much as 300 to 400 pounds. The food of these reptiles consists chiefly of vegetation and fruit. The method of food-getting and taking water furnishes ready entrance for any parasite that can survive atmospheric conditions for varying periods of time. The resistant stages of coprozoic Protozoa are also ingested and proved to be a serious handicap in our attempts at cultivation of the parasitic Protozoa. Nematodes were present in the feces in great numbers.

Species of *Nyctotherus* have been described from many vertebrates, especially frogs and toads. Daday (1905) described *N. piscicola* from a fresh-water fish *Piaretus brachypomus* of South America and later Walker (1909) described *N. multisporeiferus* from a guinea pig *Cavia cobaya*. In the class Reptilia, Grassé (1926) described *N. haranti* from the gecko, *Tarentola mauritanica* and Fantham (1929) observed and recorded the presence of *Nyctotherus* with *Chilodon* and *Balantidium* from the rectum and cloaca of one male olive water snake *Ablabophis rufulus* from South Africa. Johnston (1923) described *N. trachysauri* from the rectum of a so-called stumpy-tailed or sleeping lizard, *Trachysaurus rugosus*, of South Australia. Recently Carini (1933) described four new species of *Nyctotherus* from the posterior part of the intestine of cobras from Brazil, namely, *N. boipevae* from *Ophis meremmi*, *N. jaegeri* from *Lyophis jaegeri*, *N. coralli* from *Pseudoboa rhombifera* and *N. amarali* from *Leimadophis poicilogyrus*. This is the first account of representatives from the genus *Nyctotherus* found in tortoises. For a fairly complete list of the described species of *Nyctotherus* in both vertebrates and invertebrates see Zelif (1933). The list is brought up to date by Wicterman (1937).

Highly significant in *Nyctotherus kyphodes* and *N. teleacus* described above, are the similarities in some of the characters of the trophozoites and cysts. The peculiar ciliated and invaginated chamber which has to do with the physiological processes of excretion and egestion appears to be a new structure for ciliates. Even though the cysts of these two species have characteristic size ranges, the color of the cystic wall is almost identical and an operculum is present in both.

Unlike the genus *Nyctotherus*, species of *Endamoebae* have been described from the genus *Testudo* and related genera of turtles. Hartmann (1910) described *Entamoeba testudinis* from *Testudo graeca* but without giving a description of cystic stages. Alexeieff (1912) identified and figured *E. testudinis* from a Ceylon turtle. Wenyon (1926) also found this species in two turtles, *Testudo argentina* and *T. calcarata*. Sanders

and Cleveland (1930) mention finding *E. testudinis* in the turtle *Chrysemys picta*, cultivating it for a short time, and failing to obtain encystment. *Endamoeba barreti* was described from a snapping turtle, *Chelydra serpentina*, by Taliaferro and Holmes (1924). Sanders and Cleveland (1930) described the morphology and life cycle of *Entamoeba terapinae* from the terrapin, *Chrysemys elegans*. Knowles and Das Gupta (1930) gave a description of an *Entamoeba* sp. from a Gangetic turtle, *Trionyx gangeticus*. Fantham (1932) described *Iodamoeba testudinis* from *Testudo angulata* in South Africa but did not find cysts or figure the organism.

A careful search and comparison of described species of Endamoebae from turtles has failed to reveal a species that has a cystic stage similar to that of *Endamoeba insolita*. Sanders (1931) mentions and figures cysts of the cultivated strain of *Entamoeba ranarum* with "a jelly-like, irregular outer wall." However, the size range and other cystic characters of the two species do not agree, hence, we believe that *E. insolita* is a good morphological species. The method of locomotion and the large eccentric karyosome in the nucleus resembles the *coli* type, whereas the thin peripheral nuclear chromatin and the formation of four cystic nuclei are characteristic of the *histolytica* type in the genus. Further studies of the life-cycle will have to be pursued before the final affinities of the species can be determined.

In conclusion, we wish to suggest examination of more Galapagos tortoises, wherever available, for parasitic Protozoa. Although we were unaware of the suggestion at the time this work was started, Metcalf (1929) in a highly stimulating paper, originally suggested the examination of "Giant Tortoises in Galapagos Islands and in Malaysia" by the host-parasite method. He believed that the information gained would possibly aid in tracing east and west routes of dispersal of species in the Southern Hemisphere. The degree of specificity of parasitic Protozoa in turtles has not been tested, but since no similar Protozoa have been previously described, it is believed that at least the three new species we have described represent the parasitic fauna of these hosts in their natural habitat.

REFERENCES

- Alexeieff, A. 1912.—Sur quelques protistes parasites intestinaux d'une tortue de Ceylon (*Nicora trijuga*). Zool. Anz., 40: 97-105.
- Benedict, Francis G. 1932.—The Physiology of Large Reptiles. Carnegie Institution of Washington. p. 355.
- Carini, A. 1933.—Sobre alguns *Nyctotherus* do intestino de cobras do Brasil. Revista Biol. e Hyg., 4: 7-9.
- Chagas, C. 1911.—Sobre as variações Ciliacas do Cariozoma em duas especies de Ciliados parazitos. Contribuição para o estudo nucleo nos infuzorios. Mem. Inst. O. Cruz, 3: 136.
- Daday, E. 1905.—*Nyctotherus piscicola* n. sp. ein neuer Fisch-parasit aus Südamerika. Zool. Anz., 39: 233-238.

- Ditmars, Raymond L. 1907.—The reptile book, Doubleday, Page & Company, 1-472.
- Drbohlav, J. J. 1925.—Une nouvelle preuve de la possibilité de cultiver *Endamoeba dysenteriae*, type *histolytica*, Ann. Parasit. Hum. et Comp., 3: 349-357.
- Fantham, H. B.—1929.—Some parasitic Protozoa found in South Africa. XII. South African Jour. Sci., 26: 386-395.
- Fantham, H. B. 1932.—Some parasitic Protozoa found in South Africa XV. South African Jour. Sci., 29: 627-640.
- Geiman, Q. M. and H. L. Ratcliffe. 1936.—Morphology and life-cycle of an amoeba producing amoebiasis in reptiles. Parasit., 28: 208-228.
- Grassé, P. P. 1926.—Sur quelques *Nyctotherus* (Infusoires hétérotriches) nouveaux ou peu connus. Ann. Protistologie, 1: 55-68.
- Hartman, M. 1910.—Ueber eine neue Darmamoeba, *Entamoeba testudinis* n. sp. Mem. Inst. O. Cruz, 2: 3-10.
- Hegner, Robert. 1934.—Specificity in the Genus *Balantidium* based on size and shape of body and macronucleus with descriptions of six new species. Amer. Jour. Hyg., 19: 38-67.
- Higgins, H. T. 1929.—Variations in the *Nyctotherus* (Protozoa, Ciliata) found in frog and toad tadpoles and adults. Trans. Amer. Micro. Soc., 48: 141-157.
- Johnston, T. H. 1932.—The parasites of the "stumpy-tail" lizard, *Trachysaurus rugosus*. Trans. Roy. Soc. S. Austr., 56: 62-70.
- Knowles, R. and B. M. Das Gupta. 1930.—On two intestinal Protozoa of an Indian turtle. Ind. Jour. Med. Res. 18: 97-104.
- Lucas, C. L. T. 1928.—A study of excystation in *Nyctotherus ovalis*. Jour. Parasit., 14: 161-176.
- Metcalf, Maynard M. 1929.—Parasites and the aid they give in problems of taxonomy, geographical distribution, and paleogeography. Smithsonian Miscellaneous Collections, 81: no. 8, 1-36.
- Ratcliffe, H. L. and Q. M. Geiman. 1934.—Amebiasis in reptiles, Science 79: 324.
- Sanders, Elizabeth P. 1931.—The life-cycle of *Entamoeba ranarum*, Grassi (1879). Arch. f. Protistk. 74: 365-371.
- Sanders, Elizabeth P. and L. R. Cleveland. 1930.—The morphology and life-cycle of *Entamoeba terrapinae* spec. nov. from the terrapin, *Chrysemys elegans*. Arch. f. Protistenk. 70: 267-272.
- Stein, F. R. 1859-78.—Der Organismus der Infusiensthier. Leipzig.
- Taliaferro, W. H. and F. O. Holmes. 1924.—*Endamoeba barreti* n. sp. from the turtle, *Chelhydra serpentina*; a description of the amoeba from the vertebrate host and from Barret and Smith's cultures. Amer. Jour. Hyg., 4: 160-168.
- Tuan, H. C. 1930.—Picric acid as a destaining agent for iron-alum haematoxylin. Stain Technology, 5: 135-138.
- Van Denburgh, John. 1914.—Expedition of the California Academy of Sciences to the Galapagos Islands—The Gigantic Land Tortoises of the Galapagos Archipelago. Proc. Cal. Acad. Sci. 4th Se. vol. 2: pt. 1, 203-374, pls. 12-124.
- Walker, E. L. 1909.—Sporulation in the parasitic ciliata. Arch. f. Protistenk., 17: 297-306.
- Wichterman, R. 1934.—A new protozoan parasite, *Nyctotherus cheni* n. sp. (Ciliata) from Chinese frogs. Parasit., 26: 163-166.
- Wichterman, R. 1937.—Division and conjugation in *Nyctotherus cordiformis* (EHR.) Stein (Protozoa, Ciliata) with special reference to the nuclear phenomena. Jour. Morph., 60: 563-611.
- Wenyon, C. M. 1926.—Protozoology. 2 vols.
- Zeliff, C. C. 1933.—A new protozoan from the larva of the beetle, *Osmoderma scabra*. Proc. U. S. Nat. Museum, 82: 1-3.

EXPLANATION OF PLATES

The figures were made from fresh and fixed material with the aid of a camera lucida. All figures on Plate II were made from preparations stained with Heidenhain's iron-alum haematoxylin. Figures 1-7 were drawn at a magnification approximately $\times 750$; figures 8-9, at approximately $\times 700$; figures 10-25, at approximately $\times 2350$; and figures 26-28, at approximately $\times 3000$. Plate I was reduced by one-half and Plate II by one-third in printing.

PLATE I

- FIG. 1. *Nycototherus kyphodes* n. sp. with micronucleus and large macronucleus. Note caryophore, sub-pharyngeal canal, contractile vacuole and cytopyeal structure. Mayer's haemalum and eosin. Length $181\ \mu$.
- FIG. 2. Fresh cyst of *N. kyphodes* showing faint ciliary lines, operculum, and paraglycogen bodies in center of cyst. Length $140\ \mu$ by $90\ \mu$.
- FIG. 3. Fresh cyst of *Nycototherus kyphodes* with actively moving animal in cyst. Note spherical macronucleus (micronucleus not evident), contractile vacuole, developing membranelles and ellipsoidal paraglycogen bodies. The cyst measured $150\ \mu$ by $80\ \mu$ with the cyst wall slightly over $2\ \mu$ thick. Organism inside cyst measured $88\ \mu$.
- FIG. 4. A transverse section of *N. kyphodes* through the nuclei, cytopharynx, and groove above cytopharynx. Sectioned at $6\ \mu$ and stained with Heidenhain's iron-alum haematoxylin.
- FIG. 5. Living *Nycototherus teleacus* n. sp. showing the triangular macronucleus and clear oval micronucleus. Note caryophore, oral bristle, sub-pharyngeal canal and cytopyeal structure near the contractile vacuole. Length $127\ \mu$.
- FIG. 6. Fresh cyst of *N. teleacus* showing operculum at extreme end, ciliary lines and densely packed paraglycogen bodies in center. Length $96\ \mu$ by $58\ \mu$.
- FIG. 7. Fresh cyst of *Nycototherus teleacus* with actively moving individual within. Macronucleus and micronucleus evident; also folds in pellicle and very faint cilia. Length $96\ \mu$, width $55\ \mu$, with cyst wall $1.3\ \mu$ thick.
- FIG. 8. *Balantidium testudinis*, Chagas 1911. Note cytostome, macronucleus, micronucleus, contractile vacuole and cytopyege. Mayer's haemalum and eosin.
- FIG. 9. A cyst of *B. testudinis*. The macronucleus is smaller than in the trophozoite and the micronucleus is not evident. Heidenhain's iron-alum haematoxylin.

PLATE I



PLATE II

FIGS. 10-25 of *Endamoeba insolita* n. sp.

FIG. 10. Large trophozoite. Nucleus contains an eccentric karyosome and peripheral chromatin that is finely granular.

FIG. 11. Trophozoite containing vegetable debris. Spoke radii are evident in the nucleus.

FIG. 12. Trophozoite. Note concentric karyosome with no halo and the fine, evenly distributed peripheral chromatin.

FIG. 13. A binucleate trophozoite with spoke radii of chromatin granules in nuclei.

FIG. 14. Variation of nuclei in six trophozoites showing degeneration of chromatin to form plaques against the nuclear membrane.

FIG. 15. Trophozoite apparently in the precystic stage with cytoplasm containing bacteria and deeply staining spicules resembling chromatoids.

FIG. 16. Precystic trophozoite with bacteria in cytoplasm. The nucleus is above normal size and the karyosome is surrounded by a halo.

FIGS. 17-25. Cystic stages of *E. insolita*.

FIG. 17. Late stage of cyst formation showing chromatoids and large glycogen vacuole. Cyst wall is not completely formed.

FIGS. 18-19. Uninucleate cysts. Both cysts contain glycogen vacuoles and chromatoids show extremes of size and shape. Note irregular, thick, and typical cyst wall.

FIG. 20. A cyst with nucleus dividing. Evidence of a double cyst wall is apparent.

FIGS. 21-22. Binucleate cysts with and without a glycogen vacuole.

FIG. 23. Cyst showing division of two nuclei to form a quadrinucleate cyst.

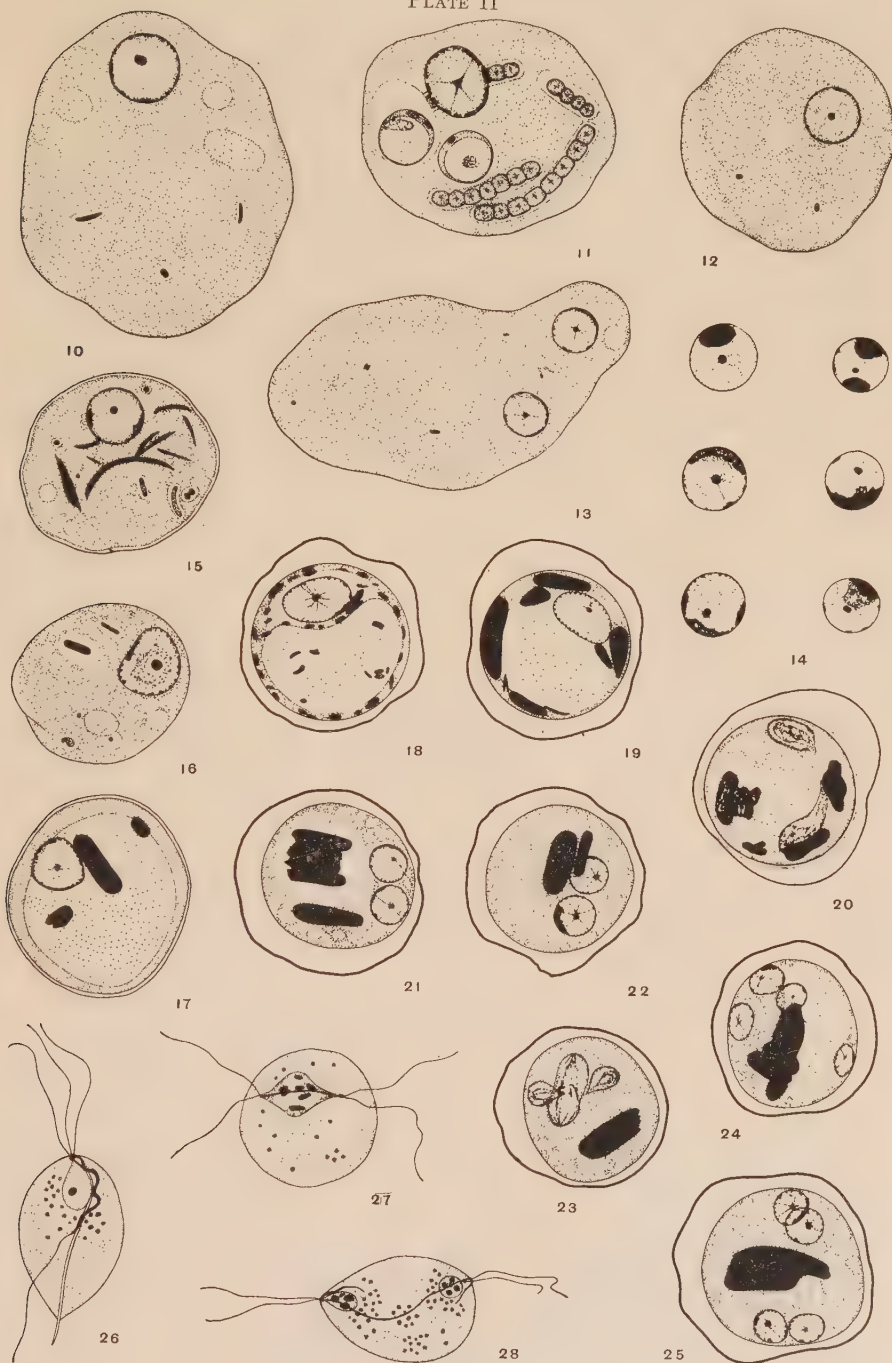
FIGS. 24-25.—Quadrinucleate cysts containing irregularly shaped chromatoids. Fig. 25 contains no glycogen vacuole and represents the typical structure found most frequently in quadrinucleate cysts.

FIGS. 26-28. *Trichomonas* sp.

FIG. 26. A typical trophozoite of *Trichomonas* sp. Note number of flagella and length of undulating membrane.

FIGS. 27-28. Division stages of *Trichomonas* sp.

PLATE II



THE IMMUNOLOGICAL SPECIFICITY OF A POLYSACCHARIDE FRACTION FROM SOME COMMON PARASITIC HELMINTHS*

DAN H. CAMPBELL

In a recent report¹ the author described the isolation of a colloidal carbohydrate from *Ascaris lumbricoides* (of swine) which gave immunological reactions in animals artificially immunized with homologous whole worm material. This substance was found to be immunologically specific with respect to a similar fraction isolated from *Monezia expansa*. Other helminths were not available at the time of the previous publication, but have since been secured in sufficient quantities to make a study of the immunological specificity of such polysaccharide fractions from seven different forms.

MATERIALS AND METHODS

The polysaccharide fractions described herein were isolated from the following helminths: (1) *Parascaris equorum*, which were secured in a fresh condition from horses in a neighboring abattoir; (2) *Toxocara canis*² which were removed from sacrificed dogs immediately after death; (3) *Ascaris lumbricoides*³ of man, which were secured in Central America where they were put into glycerine and shipped to this laboratory⁴; (4) *Ascaris suum*³ of swine, which were secured in a fresh condition from a nearby packing plant. (5) *Macracanthorhynchus hirudinaceus*, the spiny-headed acanthocephalid worm of hogs, small numbers of which were always collected along with *A. suum*; (6) *Cysticercus fasciolaris*, the larval form of *Taenia taeniaeformis* from rat livers (dried whole worm material and the polysaccharide fraction left from a previous investigation⁵ were utilized in the present study); (7) *Monezia*

* From the Department of Hygiene and Bacteriology, the University of Chicago.

This investigation was aided in part by the Logan Fund of the University of Chicago and in part by a grant from the Rockefeller Foundation to the University of Chicago.

¹ D. H. Campbell, Jour. Inf. Dis., 59: 266, 1936.

² A few worms chosen at random and carefully examined proved to have the characteristics of the Genus, *Toxocara*. It is possible, however, that some of the worms included in such samples were *Toxascaris* since the two are very similar morphologically.

³ The author has been unable to find any generally accepted systematic distinction between the common ascaris of man and of swine. The classification used here has been chosen merely to facilitate description. *A. suum* of the present report was called *A. lumbricoides* of hogs in the original report.¹

⁴ The author is indebted to Dr. D. M. Molloy of the Rockefeller Foundation, International Health Division, for securing this material.

⁵ Campbell, D. H., Amer. Jour. Hyg., 23: 104, 1936.

expansa, of sheep, which were obtained in a fresh condition from a nearby packing plant.

Of all the helminths studied, *A. lumbricoides* and *A. suum* were perhaps the most interesting from the standpoint of specificity. Previous serological studies by Hektoen,⁶ Schwartz⁷ and others,⁸ using protein or saline-extract fractions for test antigens, indicated that the two "types" were antigenically identical. This finding is further substantiated by the fact that no morphological difference between the types has ever been described. There is some biological difference, however, in that they apparently, under normal conditions, reach maturity only in their respective hosts. Particular attention has therefore been given in the present investigation to the polysaccharide fractions from *A. lumbricoides* and *A. suum* since the polysaccharides from these two types, as well as those from other helminths, were found to display a high degree of specificity.

Isolation and Purification of Polysaccharide. Procedures, described in the original report¹ for the isolation and purification of polysaccharide, have been used with slight modifications in the present study. The worms were washed, frozen and ground until a fine pulp was formed. About 50 grams of this pulp was suspended in 150 cc. of distilled water and heated for thirty minutes in a boiling water bath. This procedure not only coagulated most of the soluble protein material, but also destroyed the activity of autolytic enzymes which, under normal conditions, caused the rapid disappearance of polysaccharide. The suspension was then vigorously stirred with a mechanical stirrer, and when cool, was acidulated to approximately pH 4.5 with glacial acetic acid. Twenty cc. of 20% lead acetate were added, and the mixture placed in the refrigerator over night. The insoluble material was removed by centrifugation and discarded, and the solution added to 1.2 volumes of 95% ethyl alcohol. The precipitate, consisting chiefly of carbohydrate, was removed, resuspended in 0.1% NaCl solution and centrifuged for 45 minutes to remove any insoluble material. The resulting solution was usually biuret-negative. Further purification was carried out by fractional precipitation with 95% ethyl alcohol. The alcohol was carefully added until a faint but definite precipitate formed. This precipitate was always discarded because, although it consisted chiefly of carbohydrate, it was at times faintly biuret-positive. The soluble fraction was then precipitated by the addition of 1 volume of 95% alcohol, redissolved in 0.1% NaCl solution, and again centrifuged to remove any insoluble substances. This procedure was repeated until a sample of the resulting solution showed no evidence of precipitation when the pH was lowered

⁶ Hektoen, L., Jour. Infect. Dis., 39: 342, 1926.

⁷ Schwartz, B., Jour. Parasit., 6: 115, 1920.

⁸ Reviewed in W. H. Taliaferro, The Immunology of Parasitic Infections, New York, Century, 1929.

to approximately 3.0 with a solution of 10% phosphotungstic acid and 3% sulfuric acid. The yield obtained by such procedures varied with the species of helminth as well as the condition of worm material. The largest amount (4-5% of fresh material) was secured from *A. suum*, and the smallest amount (0.5% of fresh material) from *P. equorum*.

The polysaccharide fractions from different species of helminths are very similar in their qualitative chemical properties. For example, they form pure white amorphous powders which dissolve readily in hot or cold water. Different preparations from any one species often produce different degrees of opalescence which vary from an almost water-clear solution to one showing more opacity than solutions of commercial glycogen. The common color reactions for proteins and the diazo reaction are negative. They all give a color reaction with iodine and show no reducing power before acid hydrolysis. For unknown reasons, the specific optical rotation of different samples from any one species show considerable variation. All samples, however, produce a high dextrorotation which seems to vary directly with the opalescence of the solution. The $[\alpha]_{D^{23}}$ varies from approximately $+100^{\circ}$ to over $+300^{\circ}$. The different polysaccharide fractions are all immunologically specific regardless of the degree of their opalescence, but those producing the clearest solutions (lowest $[\alpha]_{D^{23}}$) react in much higher dilutions with their respective antisera than those producing opaque solutions. The relationships of these apparently different fractions are being studied at the present time.

Preparation of Antisera. Rabbit antisera were prepared against *A. lumbricoides*, *A. suum*, *T. canis* and *M. expansa*. Undried worm material was used for immunization since it produced a serum of much higher titre with respect to polysaccharide fractions than did dried worm material. (This was probably due to the loss of polysaccharide by autolysis during the process of drying.) The antigen was given in three series of intraperitoneal injections, as described in the previous report.¹ A total of approximately 1.5 grams was given to each animal. Fresh vegetables were eliminated from the diet during the process of immunization and the period of starvation before bleeding did not exceed ten hours. All sera were tested for non-specific reactions before using. The antigen for such tests was either polysaccharide from some helminth entirely unrelated to the form used for immunization or glycogen prepared from guinea pig livers by the method outlined above. (See previous report¹ for discussion of non-specific carbohydrate reactions.) Although non-specific reactions were rare when the above precautions were observed, they occurred at times, and the serum was discarded. Three to four rabbits were used for the preparation of each antiserum and sera showing no non-specific reactions were combined.

Preparation of Test Antigens and Technic of Precipitin Test. The polysaccharide antigens were merely dissolved in 0.9% NaCl solution in a series of dilutions starting with 1:100 and increasing by geometric progression (1:100, 1:200 . . . etc.). Saline extracts of whole worm material were prepared by stirring a sample of whole worm material in 0.9% NaCl solution for 30 minutes at room temperature and then centrifuging off the insoluble material. Such an extract, which was cloudy if obtained from undried worm material, could be cleared by incubating for thirty minutes in a 55 C. water bath and removing the flocculate which formed. Titres of the saline extracts were based upon the weight of worm material present in a unit volume of solution.

The ring method of precipitin test was used. Approximately 0.1 cc. of serum, which was diluted 1:2 with 0.9% NaCl, was placed in 4 × 50 mm. tubes and carefully overlaid with an equal volume of antigen solution. The tubes were allowed to stand one hour at room temperature and then placed in the refrigerator for about five hours. The recordings of the present report were made after refrigeration.

EXPERIMENTAL RESULTS

It will be seen from the data presented in table 1 that saline whole worm extracts from all ascarids studied displayed cross reactions with heterologous anti-ascarid serums. The highest degree of cross reaction occurred between *A. lumbricoides* and *A. suum*. In fact, the results obtained tend to demonstrate, as previous investigators have pointed out (*vide infra*), that these two forms are antigenically identical. The saline

TABLE 1.—The precipitin titres of saline whole worm extracts and of polysaccharide fractions from various helminths when tested with homologous and heterologous antisera

Test antigens	Antiserums against whole worm			
	<i>A. lumbricoides</i>	<i>A. suum</i>	<i>T. canis</i>	<i>M. expansa</i>
1. Whole worm				
<i>A. lumbricoides</i> ..	8,000	8,000	250	0
<i>A. suum</i>	7,000	14,000	400	0
<i>T. canis</i>	175	350	2,800	0
<i>P. equorum</i>	300	600	150	0
<i>M. hirudinaceus</i> ..	150	300	150	0
<i>C. fasciolaris</i>	0	0	0	0
<i>M. expansa</i>	0	0	0	480
				11,000
2. Polysaccharide				
<i>A. lumbricoides</i> ..	25,600	0	0	0
<i>A. suum</i>	0	25,600	0	0
<i>T. canis</i>	0	0	3,200	0
<i>P. equorum</i>	0	0	0	0
<i>M. hirudinaceus</i> ..	0	0	0	0
<i>C. fasciolaris</i>	0	0	0	0
<i>M. expansa</i>	0	0	0	6,400

whole worm extract from *T. canis* reacted with heterologous antisera, but to a much smaller degree than with its homologous antiserum. The

P. equorum saline whole worm extract also showed considerable cross reaction with the three anti-ascarid serums. (No antiserum was prepared for this form in the present series of experiments.)

In contrast to the saline whole worm extract antigens, the polysaccharide fractions gave precipitation reactions only with homologous antisera. This high degree of specificity was shown by all the ascarid polysaccharides including those of *A. lumbricoides* and *A. suum*. This finding indicates that the latter two types not only differ biologically, but also contain antigenically different polysaccharides.

The question now arises as to whether the antigenic difference between the polysaccharides of *A. lumbricoides* and *A. suum* is an inherent chemical quality of the worms or whether it is due to substances derived accidentally from their respective hosts and carried along during the process of purification. Although no antihuman or antiporcine serums have been prepared to test for such antigens, the question seems to be satisfactorily answered by the results obtained with *M. hirudinaceus* antigens. Since *A. suum* and *M. hirudinaceus* were obtained under the same conditions and from the same host, one may presume that if any antigenic substance from the host were carried over into the polysaccharide fractions it would have been made evident by a cross reaction between the polysaccharide fraction of *M. hirudinaceus* and anti-*A. suum* serum. Such a reaction was not observed (see table 1). Hence, the immunological difference between the polysaccharides must be a property peculiar to the parasites themselves. However, this difference may be indirectly dependent upon the host. Thus, the successful infection of hogs with *A. lumbricoides* might give rise to a strain identical with *A. suum*, as shown by polysaccharide specificity—or vice versa.

No cross reactions took place between the ascarids and tapeworms. The saline whole worm extract from *C. fasciolaris*, however, gave a positive reaction in dilutions of 1:480 with antiserum against *M. expansa*, but the polysaccharide fraction gave no reaction. The titre for saline whole worm extracts from *M. expansa* when tested with homologous antiserum was approximately 1:11,000 while that of the polysaccharide was about 1:6,400. These results are of interest, because in later work, when fresh worm material was used instead of dried worm material for immunization, the polysaccharide titre was much higher (1:25,000 or more) than those of whole worm saline extracts or of protein fractions.

SUMMARY

Methods are described for the isolation and purification of an antigenic polysaccharide fraction of helminths. Polysaccharides thus prepared, from *A. lumbricoides*, *A. suum*, *P. equorum*, *T. canis*, *M. hirudinaceus*, *C. fasciolaris* and *M. expansa* were tested with antisera

against *A. lumbricoides*, *A. suum*, *T. canis* and *M. expansa* by the precipitin ring test.

The specificity of the precipitation reaction is far greater with polysaccharides than with saline extracts of whole worm material. Whereas saline extracts of whole worm material gave precipitin reactions with heterologous serums, polysaccharide fractions reacted only with homologous antiserums. This specificity was observed even with the closely allied forms, *A. lumbricoides* and *A. suum*.

SOME COCCIDIA OF REPTILES FOUND IN NORTH AMERICA

ROBERT L. ROUDABUSH

Department of Zoology and Entomology, Iowa State College, Ames, Iowa

Up to the present very little or no work has been done on the coccidia of reptiles in North America. Becker (1934) lists all of the species recorded up to that time from reptiles, and in his list only one species of reptile is mentioned which is native to this country.

For the last year the author has been examining as many reptiles as possible for coccidia, and the present paper is the report of the material examined and parasites found.

In addition to the positive examinations listed under the discussion of each coccidium found, the following reptiles were examined and found negative. (Host determinations according to Ditmars, 1936.) 1 Indigo snake (*Drymarchon corais couperi*), 2 common hog-nosed snakes (*Heterodon contortrix*), 12 blue racers (*Coluber constrictor flaviventris*), 1 common water snake (*Natrix sipedon sipedon*), 1 bull snake (*Pituophis sayi sayi*), 1 brown water snake (*Natrix taxispilota*), 2 plains garter snakes (*Thamnophis radix*), 2 red-barred garter snakes (*Thamnophis sirtalis parietalis*), 1 western diamond-back rattlesnake (*Crotalus atrox atrox*), 1 Mojave diamond-back rattlesnake (*Crotalus scutulatus*), 3 snapping turtles (*Chelydra serpentina*), 6 western painted turtles (*Chrysemys bellii marginata*), 1 Blanding's turtle (*Emys blandingii*), 1 box turtle (*Terrapene ornata*), 1 collared lizard (*Crotaphytus collaris collaris*), 14 horned toads of which 2 were (*Phrynosoma douglassii hernandesi*) and 12 (*Phrynosoma solare*), 1 skink (*Eumeces septentrionalis*), and 1 swift (*Sceloporus undulatus*).

Isospora Dirumpens VAR. *americana* N. VAR.

Post-mortem examination of a bullsnake, *Pituophis sayi sayi*, taken at Ames, Iowa, in the fall of 1935, showed the presence of cysts of a coccidium in the contents of the large intestine. These cysts each contained four sporozoites and a residual body, and at first it was thought they were oöcysts of a *Cryptosporidium*. Further study showed the presence of two of the cysts enclosed in an outer membrane, thus proving that the cysts were really spores broken out of the oöcyst. Since each spore had four sporozoites the parasite was recognized as a member of the genus *Isospora*. All of the oöcysts found intact were taken from the small intestine, and all were fully sporulated when found.

The wall of the oöcysts is very fragile and adheres to the spores especially at the poles. Because of this, the size and shape of the oöcyst

varies with the size, shape, and position of the spores. There is no oöcystic residual body present. The oöcysts vary in length from 22.0–17.6 μ and in width from 14.5–10.1 μ . The mean size being $19.6 \times 11.8 \mu$.

The spores are oval and in some cases appear narrower at one end. The sporocyst is thick and colorless. Spores vary in length from 13.2–9.6 μ and in width from 11.0–8.8 μ , mean size being $11.17 \times 9.9 \mu$. Each spore contains four sporozoites and a large residual body.

Hoare (1933) described *Isospora dirumpens* from *Bitis arietans*, a puff-adder from Africa. The measurements, as given by Hoare, for the oöcysts were 15.2×11.2 – $16.8 \times 9.6 \mu$; for the sporocysts the measurements were 10.4×8.0 – $11.2 \times 9.6 \mu$. The general description of the parasite from the bullsnake is the same as that given for *Isospora dirumpens*.

At the same time the snake, mentioned above, was killed, pieces of the small intestine were fixed and sectioned. Study of this material showed the oöcysts located in the epithelium and sub-epithelium; the sub-epithelium being the more heavily parasitized. All of the parasites found in the tissues were sporulated oöcysts.

Transmission attempts were carried out on a young bullsnake, a pilot blacksnake (*Elaphe obsoleta obsoleta*) and a blue racer (*Coluber constrictor flaviventris*). Seven days later examination of the bullsnake and the pilot blacksnake proved them to be infected. The blue racer was refractive. (These snakes showed no coccidia before infection.) In both the bullsnake and the pilot blacksnake the only forms found were sporulated oöcysts.

Hoare (1933) found that when *Isospora dirumpens* was transmitted to another snake the only phases of the cycle found were sporulated oöcysts and because of this concluded that the second host was already immune to re-infection. As was pointed out above, the material under discussion showed the same condition, but it is felt that rather than proving immunity merely shows that either the endogenous cycle is so short as to allow only mature oöcysts to remain in the host or that the asexual phases of the cycle are passed in some other organ and only oöcyst development takes place in the small intestine. Unfortunately no material is available to prove either of these suppositions.

Hoare (1933) advances two reasons for finding only spores in the feces in infections with *I. dirumpens*. The first supposes that the oöcysts, developed in the sub-epithelium, break and allow the spores to escape alone into the lumen of the intestine. Secondly, he postulates that friction against lime particles and hair in the excrement of the host might break the delicate oöcyst wall. Observations on the *Isospora* of the bullsnake confirm the latter of the two suppositions. The bullsnake which first revealed the *Isospora* had been in captivity for two months and during that time it had not defecated. The isolated spores found in this

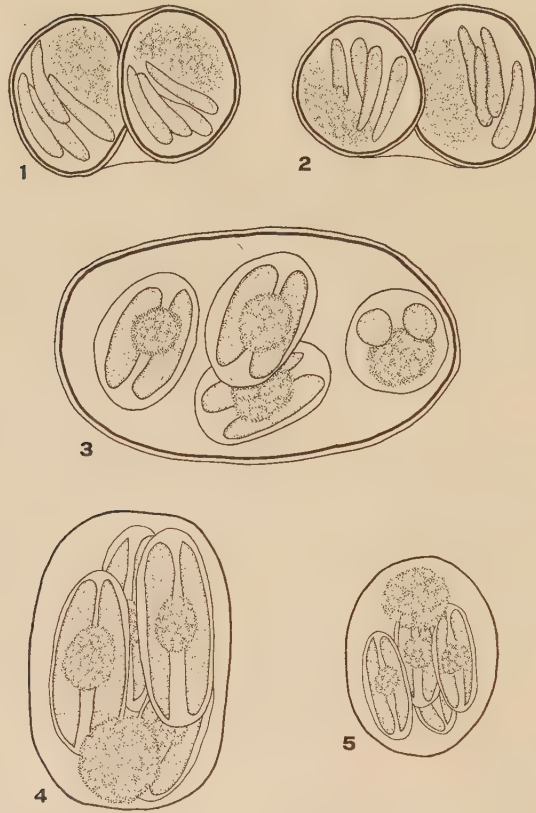


PLATE I

× 1850

FIGS. 1 and 2. *Isospora dirumpens* var. *americana* n. var. from *Pituophis sayi sayi*.

FIG. 3. *Eimeria zamenis* from *Coluber constrictor*.

FIG. 4. *Eimeria amydae* n. sp. from *Amyda spinifer*.

FIG. 5. *Eimeria dericksoni* n. sp. from *Amyda spinifer*.

snake were located in the large intestine in the retained fecal material, while all of the complete oöcysts were found in the small intestine. In other infected snakes complete oöcysts could be forced out of the large intestine for a short time after defecation.

In the spring of 1936 additional infections, with this species, were found in two bullsnakes, one pilot blacksnake, and two fox snakes (*Elaphe vulpina*), all of these being natural infections.

The *Isospora* from the bullsnake markedly resembles *Isospora dirumpens* Hoare (1933) from the puff-adder, *Bitis arietans*, but because of differences in hosts, geographical location and variations in morphology, it is believed that this *Isospora* should be considered a new

variety. For it is proposed the name *Isospora dirumpens* variety *americana* n. var.

Eimeria zamenis PHISALIX

A coccidium of the genus *Eimeria* was found in the gall bladder of three racers, one *Coluber constrictor*, one *Coluber constrictor flaviventris*, and one *Masticophis flagellum flagellum*. All of these snakes had been in captivity for some time and all had been dead for several hours before examinations were made.

The oöcysts found were elliptical in shape, and ranged in length from 35.20–30.80 μ . In width they ranged from 20.24–16.72 μ . Average size was $32.77 \times 17.81 \mu$. No oöcystic residual body was present. The spores varied from round to ovate and had a length range of 11.44–7.92 μ ; width range 9.68–7.08 μ . Mean size of the sporocyst was $9.82 \times 8.83 \mu$. A sporocystic residual body was present.

This species evidently sporulates after leaving the tissues as both sporulated and unsporulated oöcysts were present in the gall bladder. Many empty oöcysts were found in the bile—the exact reason for which is not understood, but the oöcysts may excyst in the bile and reinfect the same host.

Setna and Bana (1935) described *Eimeria flaviviridis* from the gall bladder of a lizard. This species sporulated in the bile and reinfected the host. Because of this fact it is not unlikely that the species under consideration would react in the same manner. The condition of the snakes at the time of examination made it impractical to attempt sections of the organ infected so that no comparison of the endogenous phases could be made.

Setna and Bana (1935) figure and discuss the occurrence of three spored oöcysts, one spore containing four sporozoites and the other two being normal for the genus *Eimeria*. In the material from the racers this same abnormality was encountered several times.

Phisalix (1921) described a coccidium *Eimeria zamenis* from a snake *Zamenis* sp.? In her description she gives the length range as 28–30 μ , width as 15–18 μ . The size of the sporocyst is given as 10 μ in diameter. The shape and general description of *E. zamenis* is, for all practical purposes, like that given above for the *Eimeria* from the racers. Aside from this, the genus *Zamenis* has recently been shown to be the same as the genus *Coluber*, so that it is considered that the coccidium found in the racers mentioned above belongs to *Eimeria zamenis*.

Eimeria amydae N. SP.

Examination of the fecal material in the large intestine, of a soft-shelled turtle, *Amyda spinifera*, taken at Ames, Iowa, showed the pres-

ence of coccidia of two distinct sizes. The oöcysts found were placed in 2% potassium dichromate to sporulate. The larger of the two was fully sporulated at about eighteen hours.

The oöcysts were oval and had a very thin wall composed of one layer. Inside of the oöcyst there were four spores, each containing two sporozoites and a residual body, and an oöcystic residuum. The oöcysts ranged in length from 23.66–16.72 μ ; in width from 16.72–12.32 μ . Mean size of the oöcyst was $19.55 \times 14.60 \mu$. The spores were elliptical in shape and were about two-thirds the length of the oöcyst.

Further examination of the turtle proved the oöcysts to be found in the small intestine as well as the large intestine. The gall bladder and liver were both examined but the coccidium was not found in either organ.

Becker (1934) in his host catalog of the coccidia lists only four species from turtles. Comparison was made with each one mentioned and it was found that this was a new species. For this parasite the name *Eimeria amydae* n. sp. is proposed.

Eimeria dericksoni N. SP.

The smaller coccidium from *Amyda spinifera* proved to be distinctly different from the larger one. Morphologically this species resembles *Eimeria legeri* Simond (1901) but that species was found in the gall bladder and bile ducts of *Cryptopus granosus* (= *Emyda granosa*). The description of *E. legeri* does not contain measurements, etc. so that size cannot be compared. Careful examination of the soft-shelled turtle *Amyda spinifera* showed that the coccidium did not occur in the gall bladder or in the bile ducts.

The oöcysts were usually sub-spherical in outline, but some specimens after sporulation were distorted by the position of the spores. The wall of the oöcyst was composed of only one layer and was very thin. An oöcystic residual body was present. Spores were usually elliptical in outline and contained two sporozoites and a residual body. Stieda bodies were quite evident on some specimens. The sporulation time for this species was slightly over twenty-four hours. Length of the oöcysts ranged from 16.72–12.32 μ ; width ranged from 15.84–10.56 μ . Mean size of the oöcysts was $14.55 \times 12.88 \mu$. The length of the spore is about one-half of that of the oöcyst.

This parasite is considered to be a new species and the name *Eimeria dericksoni* n. sp.* is given to it.

Hoare (1933) discusses the occurrence of thin-walled oöcysts of coccidia and says, "The facts recorded seem to indicate that the type of

* The author takes pleasure in naming this species after Dr. S. H. Derickson, of Lebanon Valley College, whose encouragement and diligent teaching was the incentive for beginning a scientific career.

oöcyst produced by coccidia depends on the site where its development takes place. It is thin walled when the sporogony is completed within the tissues of the host, and thick walled when the final stages of sporogony occur either in the lumen of the intestine or outside the body of the host." The discussion then cites the thick and thin walled *Iso-spora bigemina* as examples. This statement of Hoare's is no doubt exact in consideration of the material mentioned. An exception will be noted, however, in that the oöcysts of the two species of *Eimeria* from the soft-shelled turtle have very thin walls and that the development takes place outside of the host.

Two specimens of *Phrynosoma solare*, horned toads, were found to be parasitized by coccidia but the oöcysts did not sporulate. Since the genus could not be determined, nothing can be done but mention the fact that they were found.

LITERATURE CITED

- Becker, E. R. 1934.—Coccidia and coccidiosis of domesticated, game and laboratory animals and of man. Collegiate Press Inc., Ames, Iowa.
- Ditmars, R. L. 1936.—The Reptiles of North America. Doubleday, Doran & Co., Inc., New York.
- Hoare, C. A. 1933.—Studies on some new ophidian and avian coccidia from Uganda, with a revision of the classification of the Eimeriidea. *Parasitology*, 25: 359-388.
- Phisalix, M. 1921.—Coccidiose des serpents. *Bull. Soc. Path. Exot.*, 14: 82-84.
- Setna, S. B. and Bana, R. H.—1935.—*Eimeria flaviviridis*, n. sp., from the gall-bladder of *Hemidactylus flaviviridis*. *Jour. R. Micr. Soc. (Series 3)*, 55: 256-260.
- Simond, P. L. 1901.—Note sur une Coccidie Nouvelle, *Coccidium legeri*, Parasite de *Cryptopus granosus* (*Emyda granosa*). *C. R. Soc. Biol.*, 53: 485-486.

A FURTHER REPORT ON THE PARASITES OF A SELECTED GROUP OF EQUINES IN PANAMA

A. O. FOSTER AND PEDRO ORTIZ O.*

From the Gorgas Memorial Laboratory, Panama, R. P.

During 1935, the writers made a quantitative study of the parasites of 48 equines owned by the Panama Canal Department of the U. S. Army (2). In 1936, a second series of 49 equines was made available from the same source. This was at once appreciated as an excellent opportunity to check upon the earlier findings as well as to augment materially the data upon certain interesting interpretations (3). Accordingly, this second group was studied in the same manner, except that detailed studies were made upon the Strongylid parasites in but 38 cases, as it was apparent that the findings already published were accurately representative of the true situation and the value of additional studies seemed doubtful. On the other hand, the data on the extra-*Strongylidae*, except *Probstmayria vivipara*, were completed for the entire group, bringing the number of animals in this series to 97, with quantitative studies on the *Strongylidae* of 86. Since these studies appear to be the first to study in detail a representatively large series of equines it has seemed desirable to summarize the data upon the entire group. This has been done in Tables 1 and 2 and Graph 1.

When studied in conjunction with the first report these data appear scarcely to require explanation. It may be noted that the number of Strongylid species has been increased from 32 to 34 by the recovery of *Cyathostomum tetracanthum* and *Oesophagodontus robustus*. The former was encountered once in the dorsal colon of a horse and the latter (5 specimens only) was taken from the dorsal colon of mules on three occasions. To the extra-*Strongylidae* have been added two tapeworms, *Anoplocephala magna* and *Paranoplocephala mamillana*, each of which occurred once in mules.

These records make it evident that the parasitic infestations of imported equines of the Canal Zone are not qualitatively different from those of native stock (1). The only difference in this respect is that *C. tetracanthum* has not been encountered in native animals, while *C. metatami* has not been taken from imported equines, thus leaving the number of species the same from both groups. This similarity of parasitic fauna in two fairly isolated groups of equines suggests the probability of a cosmopolitan distribution of most of the equine species.

* The writers wish to record their indebtedness to Lt. Col. C. W. Greenlee, V. C., U. S. A., whose continued cooperation made this study possible and to acknowledge the technical assistance of Sr. Virgilio Ponce M.

TABLE I.—Summary of quantitative data on the Strongylidae of 86 equines of the Panama Canal Zone

Species	Number infested	Percentage infested	Cecum	Ventral colon	Dorsal colon	Total	Preferred localization and percent concentration
<i>Strongylus equinus</i>	69	80%	498	178	2	678	C.—73%
<i>S. edentatus</i>	65	76	68	484	6	558	V.C.—87
<i>S. vulgaris</i>	86	100	7,137	245	41	7,423	V.C.—96
<i>Triodontophorus serratus</i>	36	42	83	88	3	124	V.C.—71
<i>T. minor</i>	69	80		971	113	1,084	V.C.—89
<i>T. tenuicollis</i>	13	15		36	6	42	V.C.—86
<i>T. brevicauda</i>	13	15		20	2	22	V.C.—91
<i>Oesophagodontus robustus</i>	3	3			5	5	D.C.—100
<i>Craterostomum mucronatum</i>	5	6			5	5	D.C.—100
<i>Cyathostomum tetracanthum</i>	1	1			1	1	D.C.—100
<i>C. coronatum</i>	72	84	3,381		8	3,568	D.C.—95
<i>C. labiatum</i>	52	60	179	1,696	20	1,723	V.C.—98
<i>Gyaloecephalus capitatus</i>	57	66	7	1,229	20	1,273	V.C.—97
<i>Poteriostomum imparidentatum</i>	9	10	24	15		15	V.C.—100
<i>P. ratzii</i>	13	22			105	105	D.C.—100
<i>Cylicobrachyus brevicapsulatus</i>	25	29			90	91	D.C.—100
<i>Cylicocercus catinatus</i>	2	2			2	2	D.C.—100
<i>C. goldi</i>	77	90	111	5,481	142	5,734	V.C.—96
<i>C. vateratus</i>	76	88	12	13	2,925	2,950	V.C.—99
<i>Cylicodontophorus euproctus</i>	56	65	4	1,489	22	1,515	V.C.—98
<i>C. bicorporatus</i>	10	12			41	41	D.C.—100
<i>C. ultrajectinus</i>	12	14			25	25	V.C.—84
<i>Cylicostephanus calicatus</i>	9	10			4	4	D.C.—100
<i>C. poculatus</i>	72	84	1,153	10,308	20	11,592	V.C.—89
<i>C. minutus</i>	7	8	44		131	14	C.—100
<i>C. longibursatus</i>	72	84	44	6,024	72	6,140	D.C.—98
<i>C. hybrius</i>	81	94	6	127	15,096	15,229	V.C.—99
<i>C. asymmetricus</i>	14	16	2	60		62	V.C.—97
<i>Cylicocyclus radiatus</i>	5	19		1	6	7	D.C.—86
<i>C. elongatus</i>	16	19		41	1	42	V.C.—98
<i>C. nassatus</i>	8	9	5	5	1	11	C. & V.C.—91
<i>C. insignis</i>	75	87	491	17,883	396	18,770	V.C.—95
<i>C. leptostomus</i>	58	67	14	229	5,894	6,137	D.C.—96
<i>C. leptostomus</i>	44	51	15	478	10	503	V.C.—95
Total	86	100%	13,019	47,302	25,190	85,511	
No. different species	..	...	19	26	31	34	
Average	{ Number worms		151	550	293	994	
	{ Number species		5	11	5	15	

TABLE 2.—Summary of quantitative data on the extra-Strongylidae of 97 equines of the Panama Canal Zone

Species	No. infested	Percentage	Ave. no worms
<i>Oxyuris equi</i> ¹	32	33%	14
<i>Probstmayria vivipara</i> ²	57	66	..
<i>Parascaris equorum</i>	12	12	1 +
<i>Habronema muscae</i>	72	74	21 +
<i>H. megastoma</i>	8	8	4
<i>H. microstoma</i>	60	62	15 +
<i>Setaria equina</i>	18	19	..
<i>Anoplocephala magna</i>	1	1	..
<i>A. perfoliata</i>	1	1	..
<i>Paranoplocephala mamillana</i>	1	1	..

Ave. no. different species per animal—3.

Ave. no. extra-Strongylid worms per animal—55 +.

¹No. infested based upon the finding of either adults or 4th. stage larvae. No. worms based upon adults.

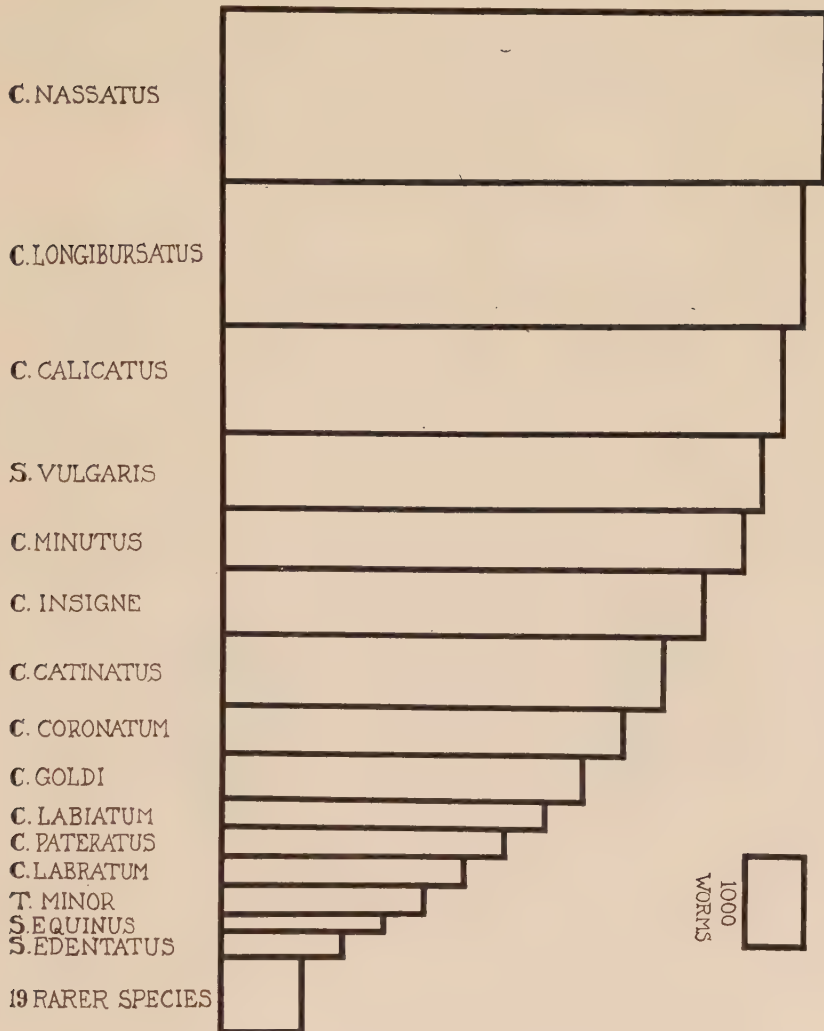
²Determined from 86 equines.

It seems neither practicable nor necessary to review the confirmations which the additional autopsies have given to the earlier interpretations. It is recognized that the data of the two reports, because of their different arrangement, do not permit of ready comparison, yet it is apparent that these data are accessible for rearranging in any desired order and are sufficiently complete, in the present report, to permit of all important comparisons. The so-called "commoner" species remain the same, as do the "rarer" ones, except that to the latter have been added the two Strongylid species mentioned above. It may be calculated from the data that the 15 "commoner" species accounted for over 98 per cent of the Strongylid parasites, a point which was emphasized in the earlier study. The quantitative differences among the 34 species are readily appreciated from a study of Graph 1. Moreover *Strongylus vulgaris* has continued to be the only species encountered at every autopsy, and *Cylicocycylus leptostomus* persists in being a "border-line" species between the "commoner" and "rarer" forms. The average infestation for the series is higher (994 Strongylid worms) than that given previously (849) but this is accounted for by the greater proportion of younger animals in the second group, and the data have shown clearly that the younger animals harbored the heavier worm burdens (3). Finally, it is felt that such quantitative variations as do exist are in the direction of increased accuracy in accordance with a substantial increase in the data.

To summarize the important findings on the 97 equines of this series the following facts may be reviewed:

1. Thirty-four species of *Strongylidae* were recovered, of which 15 accounted for about 98% of the Strongylid parasites. Fifteen was also the average number of species per animal.

2. Nineteen species occurred at one time or another in the cecum, but the average was 5. The 5 commonest species in this region accounted for 97% of the fauna viz., *S. vulgaris*, *C. coronatum*, *C. calicatus*, *S. equinus*, and *C. nassatus*.



Graph 1.—A representation of the relative abundance of the 34 species of Strongylidae from 86 equines. The data are given in Table 1.

3. Twenty-six species occurred in the ventral colon, but the average was 11. The 11 commonest accounted for about 98% of the fauna, viz., *C. nassatus*, *C. calicatus*, *C. minutus*, *C. catinatus*, *C. labiatum*, *C. pateratus*, *C. labratum*, *T. minor*, *S. edentatus*, *C. leptostomus*, and *S. vulgaris*.

4. Thirty-one species occurred in the dorsal colon, but the average was only 5. The 5 commonest accounted for over 97% of the fauna, viz., *C. longibursatus*, *C. insigne*, *C. goldi*, *C. nassatus*, and *C. catinatus*.

5. Ten species of extra-*strongylidae* were recovered, although 3 was the average per animal. The 3 commonest were *Habronema muscae*, *H. microstoma*, and *Probstmayria vivipara*.

6. No trematodes, lung-worms or "bots" were found.

REFERENCES

- (1) Foster, A. O. 1936.—Parasitic worms of equines in Panama. Proc. Helm. Soc. Wash., 3: 59-60.
- (2) 1936.—A quantitative study of the nematodes from a selected group of equines in Panama. Jour. Parasit., 22: 479-510.
- (3) 1937.—A relationship in equines between age of host and number of Strongylid parasites. Amer. Jour. Hyg., 25: 66-75.

A NEW SPECIES OF *ORNITHODOROS* TICK FROM
CALIFORNIA (ACARINA, IXODOIDEA)*

BARBARA CATHERINE McIVOR

Ornithodoros wheeleri n. sp.

ADULT FEMALE:

Body: Ovate-oblong, somewhat narrowed and with margin broadly rounded anteriorly, hood visible dorsally. Integument medium thick, usually sandy in color when unengorged. Freshly engorged specimen a deep garnet brown, color changing to a blue-gray a few days after feeding. True discs absent, unmammilated tracts present, appearing as furrows and depressions in unengorged specimen. Numerous long club-shaped hairs present, arising individually from some of the mammillae, also scattered stylete hairs arising from individual mammillae. Microscopically, integument irregularly mammillated, mammillae surrounded by a



Fig. 1. *Ornithodoros wheeleri* n. sp., adult female. A, dorsal view; B, ventral view. $\times 11$.

rosette of sinuous grooves. Dorsally, sculpturing consists of well defined, bilaterally arranged depressions with a median posterior groove, anterior marginal groove broadly U-shaped. Margins of body rounded in unengorged specimen. Depressions well marked in engorged specimen, grooves less apparent.

Venter: Integument similar to that of dorsum. Eyes lacking. Coxal and supra-coxal folds well marked, supra-coxal folds extending anteriorly to form the margins of the hood, supra-coxal grooves extending laterally to coxae IV, expanding into a triangular depression surrounding the spiracle then diverging to form a notch in the lateral margin of the body. Sub-coxal folds originating at the base of coxae II, expanding and extending to coxae IV. Sub-coxal grooves meeting the genital grooves anteriorly and joining the supra-coxal grooves poste-

* From the Division of Entomology and Parasitology, University of California, Berkeley, California.

rior to the fourth pair of coxae. Median post-anal groove a well marked tri-undulate groove. The transverse post-anal groove biundulate, following the contour of the pre-anal groove, with the anterior margins thickened and somewhat granular in appearance.

Anus: Ovate, about 295.0 microns long and 290 microns broad.

Spiracles: Located in triangular expansions of the supra-coxal grooves dorsal to coxae IV. Spiracle circular, with a crescentic plate, width about 180 microns.

Hood: Short, curved ventrally, broadly pointed anteriorly, many long hairs on the ventral surface.

Capitulum: Placed in a deep camerostome, with few hairs.

Hypostome: Slightly lanceolate, of fair size, approximately 400 microns in length and 120 microns in width, apex emarginate. Dentition: Crowned with three or four semi-circular rows of minute teeth, followed by two semi-circular rows of slightly larger teeth and one row of fairly large teeth. The principal dentition consisting of two bilateral rows of four large teeth. Posterior to these are three bilateral rows of three somewhat smaller teeth. There follows a triangularly arranged group of about 13. A triangular protuberance projects from the lateral margins of the hypostome 228 microns from the apex. Two long, stout, *post-hypostomal* hairs present on the ventral surface of the capitulum.

Chelicerae: Digit approximately 100 microns long. External article tridentate, denticles increasing in size proximally. Internal article rounded at the apex, bearing a bidentate process near the base. Cheliceral sheaths with a broad medial band of many rows of minute teeth.

Palpi: Comparatively long—Articles I–IV, measuring 332 microns, 208 microns, 133 microns and 133 microns respectively. Article I bearing a clump of four long bristles on the inner aspect of the distal end and a patch of stout bristles on the dorsal surface. Article II with numerous medium and long bristles, with few serrate bristles on the inner surface. Article III with few scattered variable bristles, bearing one or two serrated bristles on the inner aspect. Tip of article IV broadly rounded, equipped with two sets of spines, one clump of three long spines and a second clump of seven short spines.

Legs: Long and slender, coxae progressively smaller from I to IV. Tarsus I equipped with five well defined diagnostic protuberances with a club-shaped hair present between the basal two; the two anterior protuberances separated by Haller's organ. Tarsus IV equipped with one well defined protuberance above the claws, claws surrounded by six long spines, one lanceolate spine present at the apex of the distal protuberance, numerous blunt and lanceolate hairs present on the tarsus.

Proportions: Female, length, 5 to 7 mm.; width, 3.5 to 5 mm. Male similar to female although somewhat smaller in size, length 3 to 4.5 mm.; width 2 to 3.5 mm.

Holotype, female, taken at Los Banos, Merced County, California, October 12, 1935, on man. Allotype, male, reared from eggs of the holotype female. Holotype deposited in the collection in the Division of Entomology and Parasitology, University of California, Berkeley.

This new species differs from *Ornithodoros turicata*, a near relative, in having (a), the club-shaped hairs much longer, (b), the thickening of the anterior margins of the transverse post-anal groove, (c) the more pointed capitulum, (d), a distinctive arrangement in regard to the number of teeth on the hypostome, and (e), the arrangement and number of protuberances on Tarsi I and IV in particular. This new tick differs from *Ornithodoros parkeri* in (a), in the size of the mammillae; and (b), the arrangement and number of protuberances of Tarsi I and IV.

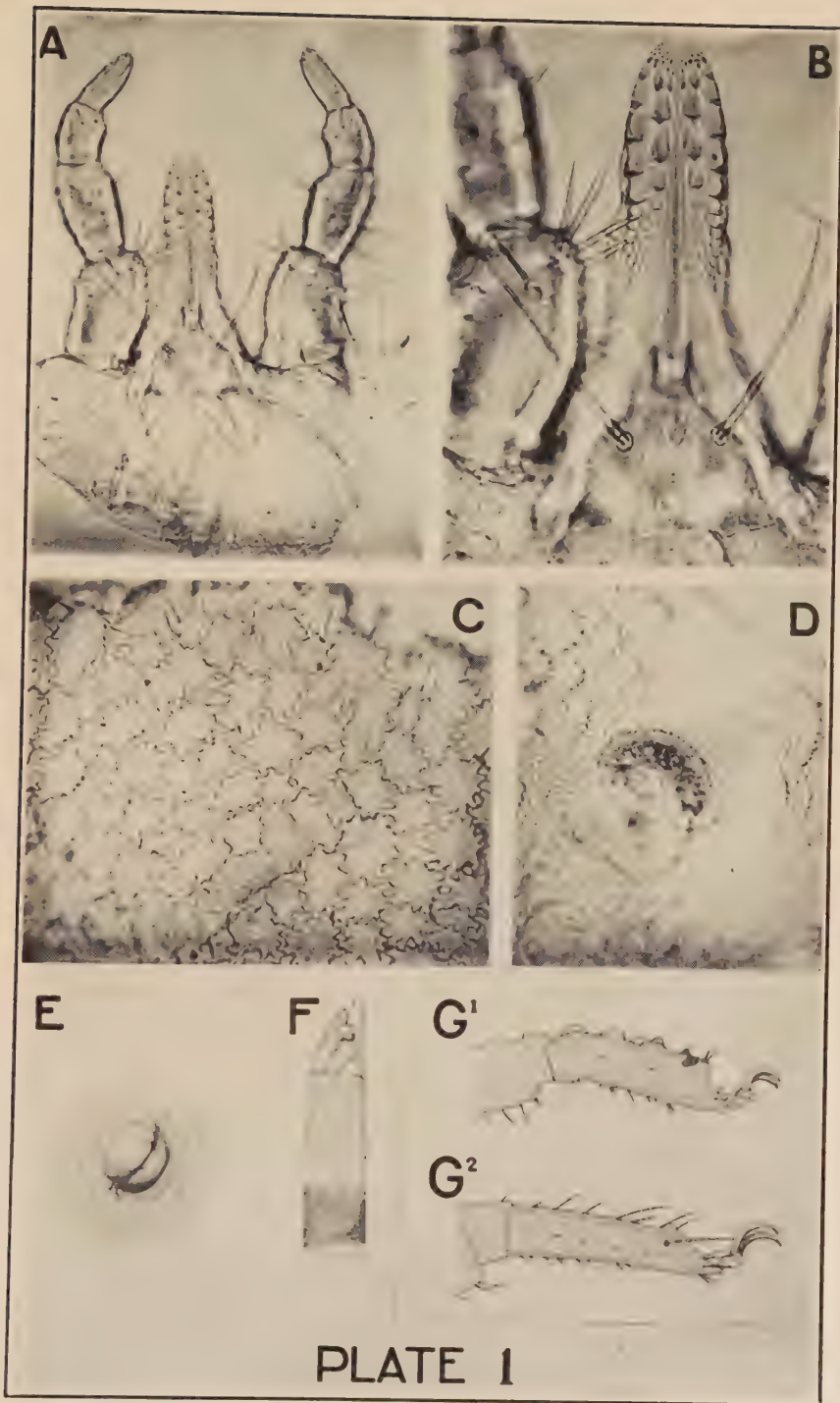


PLATE I
Ornithodoros wheeleri n. sp. A, Hypostome and palpi; B, Hypostome greatly enlarged; C, Integument; D, Spiracle; E, Anus; F, Chelicera; G¹, Tarsus of 1st leg; G², Tarsus of 4th leg.

THE VIABILITY OF THE OVA OF *ASCARIDIA LINEATA* WHEN EXPOSED TO VARIOUS ENVIRONMENTAL CONDITIONS

P. P. LEVINE

Department of Pathology and Bacteriology, New York State Veterinary
College, Ithaca, New York

The need for knowledge of the longevity of parasite eggs and larvae when exposed to various environmental conditions is obvious. It is upon such knowledge that parasite control measures, especially range management and range rotation, are based.

Comparatively little work on the viability of the eggs of *A. lineata* has been reported. The papers by Ackert and Cauthen (1931) and Ackert (1931) are the only ones known to the writer which deal with this problem.

The experiments described in this paper were designed to confirm, if possible, and extend the work of these workers.

METHODS

For testing the viability of ascarid eggs which had been subjected to various environmental conditions, the same general procedure was employed throughout. The eggs were secured from adult female worms and were embryonated by incubating them in shallow layers of distilled water for 14 to 16 days at 30° C. A suspension of the embryonated eggs was then thoroughly mixed with dry sterile silt loam soil. The moisture content of the soil was adjusted by adding more water or evaporating as the case required. The number of eggs in the soil was such that a 5 or 10 gram sample, when fed to susceptible chicks, produced at least 25 worms. Generally, the number of worms found was greater than one hundred. Non-embryonated eggs were mixed with soil in the same way and when a sample was to be tested for viability it was first incubated for 16 to 18 days at 30° C. before it was fed to chicks. White Leghorn chicks from 2 to 5 weeks old were used since it has been shown that young chicks are more susceptible to ascarid infection than older birds (Ackert, Porter and Beach 1935). These chicks were reared in batteries with wire screen floors and kept, after dosing, in sterilized cages with wire screen bottoms. The soil samples were fed to the birds either in No. 3 gelatin capsules or by pipetting a heavy soil suspension into the crop. In the following experiments viability of the eggs was determined by finding young parasites in the intestinal mucosa of the birds 11 days after they had been dosed. At that time the parasites are still in the mucosa and no diminution in their numbers has occurred. The writer

recognizes the fact that it is possible for non-embryonated ova to be viable and still be incapable of complete embryonation after proper incubation (Ackert 1931). From a practical standpoint it would not be illogical to consider such ova as dead. The method used for finding the worms was that described by Levine (1936).

EXPERIMENTAL WORK

EXPERIMENT I

Viability of the Ova of A. lineata in Soil after Exposure to Weathering

Two large clay flower pots were filled with soil to within $1\frac{1}{4}$ inches of the top. There was a hole in the bottom of each pot to allow for proper drainage. An embryonated egg-soil mixture (20 per cent moisture) was prepared and placed in these pots to form a top layer $\frac{3}{4}$ inches thick. Both pots were set in the ground deeply enough so that the top of the egg soil layer was even with the ground level. The rim of each pot protruded $\frac{1}{2}$ inch above the ground surface and so prevented the washing out of the soil egg mixture. One pot was buried in the shade of some pine trees so that the direct rays of the sun could not strike it. The other pot was buried in a place exposed to the sun's rays all day. When the eggs were to be tested for viability a soil sample was obtained by cutting a core through the soil-egg mixture so that all depths of the layer were sampled. Two trials were run 8 months apart and the results are recorded in Table I. Another pair of flower pots, containing a non-embryonated egg-soil mixture, were buried in similar locations, and the results are shown in Table 2.

The data in Table 1 show that embryonated eggs in the shade survived during the spring, summer and fall of 1935 but were killed during the winter. The fact that the ground did not freeze until after the test date in November is significant and will be referred to later. The data in the second trial show conclusively that freezing and not aging killed the eggs.¹ Exposure to the sun was rapidly fatal to the embryonated eggs. The fact that hardly any rain had fallen for three weeks previous to the first negative result probably explains this result.²

Table 2 shows that the non-embryonated eggs survived the winter despite the fact that February was the coldest month experienced in many years.³ The ova exposed to the sun died during the summer of the fol-

¹ Mean Temperatures: Nov. 21 to 30—Max. 41.2° F. Min. 30.2° F. Dec. 1 to 31—Max. 29.2° F. Min. 18.5° F. (Sub-zero once). Jan. 1 to 13—Max. 36.6° F. Min. 27.6° F.

² From May 12 to June 5 there was no rain for 17 days, less than .01 inch on 4 days and .09, .03, and .47 inches respectively on three days.

³ February 1936 had a mean Max. temperature 28.0° F. Min. -9.3° F. Sub-zero temperatures were recorded 7 days in this month.

lowing year due, in all probability, to a severe drought.⁴ The ova in the shade not only survived the summer but were embryonated when tested in August.

EXPERIMENT II

Viability of the Ova of A. lineata after Subjection to Drying

A suspension of embryonated ascarid eggs was mixed with fresh chicken feces and this was then thoroughly dried with the aid of an electric fan. The dried material was stored in the dark at room temperature. The results obtained showed that the embryonated eggs were infective after 21 days of drying but were dead when tested on the 40th day.

Two similar experiments were set up with a non-embryonated egg-soil mixture. In the first trial the non-embryonated eggs were viable after 16 days of drying but not after 64 days. In the second trial the time interval between the tests was shortened, and it was found that the eggs were viable after 44 days of drying, but not after 51 days.

EXPERIMENT III

Viability of the Ova of A. lineata in Soil Subjected to a Temperature of -1 Degree Centigrade

A suspension of freshly embryonated eggs was removed from the incubator, mixed with soil and the moisture content of the mixture adjusted to 20 per cent. The soil-egg mixture was then promptly placed in the freezing compartment (-1°C.) of an electric refrigerator, after a control sample had been removed. Two trials soon showed that the embryonated eggs were destroyed in less than 20 hours exposure. A third trial was run and this time soil-egg mixtures containing 0, 10, 20 and 40 per cent moisture respectively were used. It was found that the eggs in all samples were destroyed in less than 13 hours exposure.

Since it was apparent that rapid cooling to -1 degree C. was lethal for embryonated eggs, a more gradual cooling was effected in two other trials. In the first trial embryonated egg-soil mixtures with moisture concentrations of 0, 10, 20, and 40 per cent, respectively, were kept in a refrigerator at 10°C. for 21 days before they were placed in the freezing compartment. Tests made after 1, 3 and 5 days exposure at -1°C. showed that the ova were viable. In the second trial a soil-egg mixture (0 per cent moisture) was cooled for 11 days at 10°C. and was found to be infective at that time. However, after subsequent exposure to -1°C. for 14 days, the ova were non-viable. In the same trial two egg-soil samples of 20 and 40 per cent moisture, respectively, were cooled at 10°C. for 12 days and then frozen at -1°C. Tests made after 14 hours of

⁴ From May 11 to July 7 there was no rain for 36 days, less than .01 inch on 5 days and rain on 17 days in very small amounts and at widely separated intervals.

freezing showed the eggs to be viable, but those made after 14 days indicated that they were dead.

It is apparent from these results that preliminary cooling had the effect of increasing the survival time of the embryonated eggs exposed to freezing temperatures to a period greater than 5 but not as long as 14 days. These results harmonize with those obtained in Experiment I in which it was found that embryonated eggs exposed to weathering did not survive the winter.

Two trials were run with non-embryonated egg-soil mixtures (20 per cent moisture). Preliminary cooling was not done. In the first trial the non-embryonated eggs survived for 20 days but not for 65 days at -1°C . In the second trial the eggs survived for 45 but not for 52 days.

EXPERIMENT IV

Viability of the Ova of A. lineata in Putrefying Feces

A heavy suspension of embryonated ascarid eggs was mixed with four times its volume of fresh chicken manure and placed in a flask fitted with a one hole rubber stopper to reduce evaporation. This mixture was placed in an incubator at 30°C . and allowed to putrefy. Under these conditions the embryonated eggs survived 7 days, but were dead on the 14th day.

The same experiment was set up with non-embryonated eggs. This mixture was allowed to putrefy at room temperature. When a sample was to be tested it was mixed with an equal volume of $1\frac{1}{2}$ per cent potassium dichromate solution before incubation. This was done in order to retard the putrefaction and allow embryonation to take place. It should be noted that for the first two weeks in this trial the putrefactive process was slow in developing. In this experiment it was found that the non-embryonated eggs were viable after 88 days exposure, but were not viable when tested after 121 days.

EXPERIMENT V

Time Necessary for Embryonation of the Ova of A. lineata Under Natural Temperature Conditions in Moist Soil

Since it has been shown that non-embryonated eggs can survive severe winter weather, the question arose as to how long it would take for such eggs to embryonate the following spring. An experiment was planned to attempt to obtain information on this matter. A mixture of non-embryonated eggs and soil was prepared and placed in a flower pot in the same way as described in Exp. I. The pot was set in the ground in a shady spot so that the direct rays of the sun could not strike it. The soil-egg mixture was kept moist throughout the duration of the experiment by watering the soil daily. In this way a typical wet spot on a

range was simulated. Samples of soil were removed at intervals and tests were made for embryonation of the eggs. Two trials were run side by side and under identical conditions. The first trial was started April 11, 1936 and embryonation was found to have taken place some time between June 27 and July 17. Embryonation in the second trial which was begun on April 29, had not been completed by June 6 but was completed by July 7. In view of the fact that both trials were run exactly alike and under identical conditions, it would be reasonable to conclude that the eggs in the first trial must have completed embryonation between June 27 (the last negative test) and July 7 (the first positive test in the second trial). Ackert and Cauthen (1931) state that eggs exposed in the spring embryonated by May and June. The ova in our tests embryonated sometime during the last week in June and the first week in July. There can be no doubt that the variation in weather conditions from year to year and in different localities is responsible for the variation in the embryonation times of ascarid ova.

DISCUSSION

The observation (Ackert and Cauthen 1931) that ova in unshaded soil in summer were destroyed in three weeks, but in shaded soil remained viable from spring to autumn was largely confirmed by our results. Ackert further states: "In two inches or less of soil the eggs fail to survive normal winters (with sub-zero weather) but may live through mild winters." He does not state, however, whether these observations were made with embryonated or non-embryonated eggs. Examination of Tables 1 and 2 shows that survival of the eggs during the severe winter of 1935-36 depended on whether or not they were embryonated. The embryonated ova which were viable in November in both trials were dead when tested in January of the following year. On the other hand the non-embryonated ova survived the same winter. The non-embryonated ova which had been exposed to the sun were found to be non-viable the following July while those in the shade not only survived the summer but were embryonated when tested in August.

It is apparent from the preceding discussion that a poultry range contaminated in the fall with embryonated and non-embryonated eggs would still harbor viable non-embryonated eggs in the following spring. Since it is probable that some of these ova would embryonate in the summer another winter would have to pass before they, in turn, would be destroyed. From consideration of the results procured in Experiments I and III it would not be unreasonable to conclude that all viable ascarid ova on a range will be destroyed after two successive winters provided that the surface strata of soil remain frozen for two weeks or more during those winters.

Rapid cooling to -1°C . destroyed freshly embryonated ova after 13 hours exposure. The fact that pre-cooling at 10°C . served to extend the longevity of the eggs when subsequently frozen is an extremely interesting one from a purely biological point of view. Recent work along this line (Sherman and Cameron 1934) shows that young rapidly dividing bacterial cells (*B. coli*) suffer a 91 to 97 per cent mortality when subjected to a sudden drop in temperature from 45 to 10°C . Loeb and Wasteneys, (1912) cited by Sherman and Cameron, showed that fish (*Fundulus*) when transferred suddenly from water at 10°C . to 35°C . died rapidly. If the change in water temperatures was brought about gradually there were no untoward results. Apparently the "shocking" effect of sudden environmental changes is lethal to many forms of life. To the writer's knowledge this is the first time that such an effect has been noted with ova of parasitic nematodes. However, it must be stated that all nematode ova are not affected by a sudden temperature drop as are the ova of *A. lineata*. The writer has shown* that freshly embryonated ova of *Capillaria columbae* are unharmed by rapid cooling to -1°C . This selective action of rapid freezing can be used to eliminate viable embryonated ascarid ova from a suspension containing both embryonated *A. lineata* and *C. columbae* eggs.

Ackert (1931) has shown that non-embryonated *A. lineata* ova kept at 0°C . for one month failed to reach the infective stage after subsequent incubation. Approximately the same results were obtained by us in Experiment III in which it was found that the eggs were non-viable after 45 days at -1°C . On the other hand, it has been noted in Experiment I that the non-embryonated eggs exposed to weathering survived an extremely severe winter during which there were more than 45 days of freezing weather. We would not be justified in comparing results obtained in these two experiments since conditions were not the same. However, it is quite possible that had the non-embryonated ova in Experiment III been slowly cooled down previous to freezing, the survival time might have been greatly prolonged.

CONCLUSIONS

1. Embryonated ova of *A. lineata* in soil exposed to weathering in the shade were viable after 242 days exposure (March to November, 1935) but were not viable when tested the following January after freezing weather had set in.

Embryonated eggs in soil exposed to weathering in the sun were viable after 46 days exposure (March to May 1935) but not after 72 days.

2. Non-embryonated ova of *A. lineata* in soil exposed to weathering in the shade (November 1935) survived the winter of 1935-36 and were embryonated when tested in August 1936 (279 days).

* Unpublished data.

Non-embryonated eggs in soil exposed to weathering in the sun (November 1935) also survived the winter of 1935–36 and were still viable in May 1936 (186 days) but were not viable when tested in July (243 days).

3. Embryonated ova of *A. lineata* thoroughly dried in feces survived for 21 days, but were not viable after 40 days.

Non-embryonated eggs when dried in soil were viable after 44 days but not viable after 51 days.

4. Freshly embryonated ova of *A. lineata* in soil (0 to 40 per cent moisture) was destroyed after 13 hours exposure at -1° C. Freshly embryonated eggs when cooled for 21 days at 10° C. survived subsequent exposure at -1° C. for 5 days. Freshly embryonated eggs cooled for 12 days at 10° C. were non-viable after subsequent exposure at -1° C. for 14 days.

Non-embryonated ova in soil survived for 45 days but not for 52 days at -1° C.

5. Non-embryonated eggs in putrefying feces at room temperature were viable after 88 days but not after 121 days.

Embryonated eggs in putrefying feces at 30° C. were viable after 7 days but not after 14 days.

6. Non-embryonated ova of *A. lineata* in moist soil exposed to natural temperature conditions on April 11, 1936, were not embryonated when tested on June 27 but were embryonated on July 17. In another test started on April 29, 1936, embryonation was not complete on June 6 but was complete on July 7.

REFERENCES CITED

- Ackert, J. E. 1931.—The morphology and life history of the fowl nematode *Ascaridia lineata* (Schneider). *Parasitol.*, 23: 360.
- Ackert, J. E. and Cauthen, G. E. 1931.—Viability of the eggs of the fowl nematode *Ascaridia lineata* (Schneider) exposed to natural climatic factors. *Jour. Parasitol.*, 18: 113.
- Ackert, J. E., Porter, D. A. and Beach, T. D. 1935.—Age resistance of chickens to the nematode *Ascaridia lineata* (Schneider). *Jour. Parasitol.*, 21: 205.
- Levine, P. P. 1936.—The treatment of ascariasis in chickens. *Cornell Vet.* 26: 119.
- Loeb, J. and Wasteney, H. 1912.—On the adaptation of fish (*Fundulus*) to higher temperatures. *Jour. Exp. Zool.*, 12: 543.
- Sherman, J. M. and Cameron, G. M. 1934.—Lethal environmental factors within the natural range of growth. *Jour. Bact.*, 27: 341.

SOME HISTOPATHOLOGICAL CHANGES CAUSED BY *HYPODERMA* LARVAE IN THE ESOPHAGUS OF CATTLE

S. W. SIMMONS

Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

Numerous macroscopic observations of the esophagus of cattle infested with *Hypoderma* larvae have been made, but there seems to be a lack of detailed knowledge concerning the microscopic changes that occur. The present paper describes some of the changes brought about in the esophageal tissue of cattle invaded by the common cattle grub, *H. lineatum* DeVill.

TECHNIQUE

Material for this investigation was obtained from cattle slaughtered at an abattoir in Des Moines, Iowa. As soon after slaughter as possible tissue from infested esophagi, with larvae intact, was placed in Zenker's fixative and prepared for sectioning and staining. Paraffin was used exclusively for imbedding purposes.

Several staining techniques were used to bring out the desired effects. Eosin-methylene blue staining proved very effective for demonstrating infiltrating leucocytes. Best results were obtained by staining in a 4 percent aqueous solution of eosin Y for 20 minutes, counter-staining with Goldhorn's polychrome methylene blue for 15 minutes, and then differentiating in 95 percent alcohol containing a few drops of rosin solution (1). Picrofuchsin (Van Gieson) was an excellent counter stain to hematoxylin for differentiating connective tissue, which, owing to pathological conditions, did not always appear as expected. The ordinary Delafield hematoxylin-eosin staining was employed in some instances.

In estimating the number of eosinophils per cubic millimeter of tissue, counts were made of all the cells falling in the area of a crossline ocular micrometer. The square area of the ocular micrometer was computed for each power of the microscope, and the thickness of the tissue was known. In computing densities per cubic millimeter of tissue, the following formula was used:

$$D : Ta = N : 1$$
$$\text{or } N = \frac{D}{Ta}$$

where a = area in which cells were counted, T = thickness of the section, D = cell population of area counted, and N = calculated number of cells per cubic millimeter of tissue.

Most of the tissue examined was taken from animals late in the fall, when the larvae averaged about 10 to 15 mm. in length. Since at this time most of the specimens were situated in the posterior half of the esophagus, the tissue examined was generally from this region.

RESULTS

Tissue Changes: The usual location of *Hypoderma lineatum* in the esophagus is in the submucous layer. The invasion by the parasite causes certain local changes in the tissue, and those observed are discussed below.

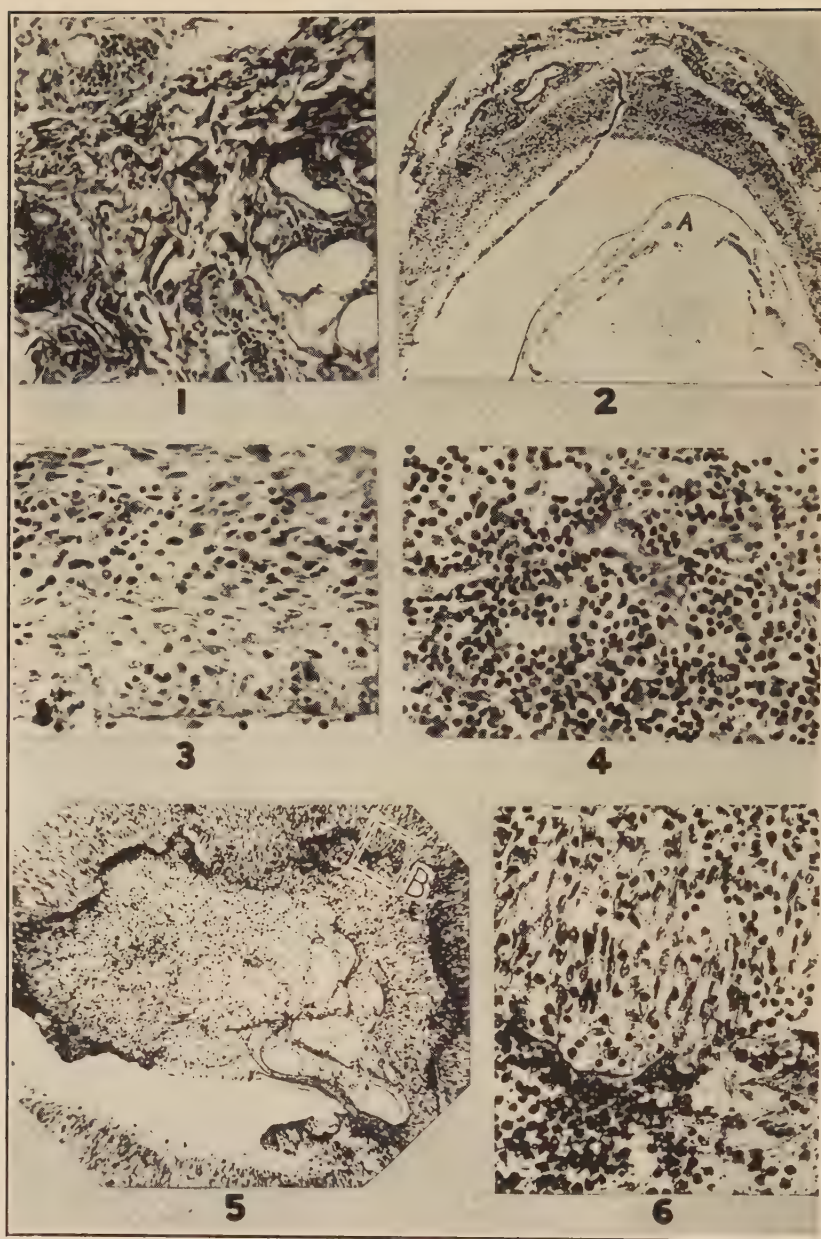
The connective tissue in the vicinity of the larvae was distorted by an inflammatory edema, which was usually extensively cellular (figs. 1 and 4). Frequently there was increased vascularity in the connective tissue about the larvae, but very little hemorrhage was seen in these areas.

Irritation by the larvae and their secretions possibly plays a part in increasing the permeability of adjacent capillaries and lymphatics, thus favoring the exudation of dropsical fluid. Cytolysis by larval toxins would tend to increase the osmotic pressure of the tissue. Such a disturbance would cause the plasma to pass from the vessels into the tissue (2). Bacterial toxins are probably excluded from the process, as Hadwen (4, 5) found that larvae from the esophagus were sterile.

The infiltrating fluid presses between, separates, and breaks up the fibrous strands of the submucosa (fig. 1). In a few cases the muscularis mucosa was invaded, and a distortion of this layer occurred. Some of the fluid in this region was noncellular. As a rule, however, little injury to the muscularis mucosa was evident. The lamina propia was rarely invaded, although this was found to happen in one or two cases. Large larvae, or several larvae in close proximity, sometimes produced a reaction sufficiently strong to create a slight pushing in of the mucous layer toward the lumen of the esophagus. In no tissue examined, where the larvae were in the submucosa, was there appreciable distortion of the main muscular layers.

The degree of infestation and the rate of larval migration affect the tissue surrounding the larva. Definite compact bands of fibroblasts were sometimes formed around the specimen (figs. 2 and 3). The thickness of these bands varied, but some measured as much as 350 microns. There was some fibroblastic formation about most of the specimens examined, but a compact band was not always present. This band was probably formed about the stationary or slowly moving specimens.

Cellular Infiltration: One of the most striking phenomena always observed was the enormous number of leucocytes present in the edematous area of the submucosa (fig. 4). The predominating type of cell was usually the eosinophil, although many lymphocytes were usually present, and plasmocytes were relatively common. Polynuclear cells other than



DESCRIPTIONS OF FIGURES IN PLATE I.*

All figures illustrated are 8 microns thick and were stained with eosin-methylene blue.

Fig. 1. Effect of edematous fluids on connective tissue of submucosa. Note severe distortion of fibrous strands and cellular infiltration. $\times 120$

eosinophils were rare. In addition there were found a few mast cells and giant cells. Macrophages in general were relatively scarce.

It is difficult to make an accurate differential analysis of cell types, or to give a quantitative estimation of the number of cells present in tissue sections. This is due to the fact that sections through different pathological regions give variable counts (tables 1 and 2), and in the tissue examined many of the cells were myelocytes and other immature forms which added to the difficulty of their determination. Variations in counts were due no doubt in part to the chronicity of the infestation, its intensity, and probably to the individual physiological reactions of the host.

Attempts were made to determine densities only of eosinophils. Table 1 gives representative densities of this type of cell from various parts of the submucosa of the esophagus of several animals.

TABLE 1.—*Some Examples of Eosinophilic Densities in Submucosal Tissue of the Esophagus in the Vicinity of Hypoderma lineatum larvae*

Total number of Eosinophils per cubic millimeter of tissue	Location of count in submucosa
385,750	In young connective tissue serosad of and adjacent to larval channel in healing area.
788,000	90.9 microns serosad of larval channel in healing area.
123,500	In connective-tissue band laterad of larva.
77,125	In connective-tissue band epitheliad of and adjacent to larva.
370,375	Adjacent to muscularis mucosa, epitheliad of healing larval channel.
509,250	At end of larva and in close proximity.
725,250	1.48 mm. from end of larva.
370,375	Adjacent to first muscle layer serosad of larva.
895,000*	Ditto.
401,250	In young connective tissue serosad of larva in healing area.
431,125	Adjacent to muscularis mucosa epitheliad of healing larval channel.
478,125	Serosad of and adjacent to larva.
637,750	18.1 microns epitheliad of first muscle layer, and 3.33 mm. obliquely serosad of larva.

* Only small areas showed densities of this magnitude.

The greatest density of eosinophils is not invariably in tissue immediately adjacent to the larva. It may be several millimeters from the

Fig. 2. Connective tissue band or pseudocyst, formed about a larva in the submucous layer of the esophagus. Remains of larvae indicated by A. $\times 44$

Fig. 3. High magnification of a section of the band shown in figure 2. Note fibroblastic formation. $\times 230$

Fig. 4. Area of submucosa in vicinity of larvae, showing infiltration of leucocytes, principally lymphocytes. $\times 230$

Fig. 5. Healing channel made by larvae in submucosa. The dark band indicates junction between young tissue and luminal debris. $\times 44$

Fig. 6. High magnification of area B in figure 5. Note perpendicular orientation of connective-tissue fibers toward debris-filled channel left by larva. $\times 230$

* The writer is indebted to Dr. A. M. Lucas, Department of Zoology, Iowa State College, for his assistance in preparing the photomicrographs.

parasite, in the submucosa. In the periphery of the pathological area there is a decrease of eosinophils to almost zero.

In these counts eosinophils were present in the tissue in an average quantity of 476,365 cells per cubic millimeter. The number varies considerably in the different regions of the same edematous area, as is shown in table 1.

An interesting phenomenon observed, especially in heavy infestations, was the large number of eosinophilic myelocytes present. Many eosinophilic areas contained only mature forms, but in some the percentage of myelocytes was relatively high. An idea of the ratio of mature to myelocytic eosinophils, where these occurred, may be obtained from table 2.

TABLE 2. *Some Examples of the Percentage of Mature and Myelocytic Eosinophils in Areas where Both Were Prevalent*

Total number of eosinophils per cubic millimeter of tissue	Mature eosinophils (per cent)	Myelocytic eosinophils (per cent)	Location of count in submucosa
478,122	96	4	Serosad of and adjacent to larva.
321,875	56	44	1.3 mm. serosad of larva.
196,875	79	21	0.2 mm. epitheliad of larva.
100,000	79	21	Laterad of and in close proximity to larva.
895,000	75	25	Adjacent to first muscle layer serosad of larva.

The presence of the myelocytic forms in the percentages shown indicates the heavy demand on the haemapoietic organs for the production of cellular elements. The myelocytic eosinophils present may be from zero to 44 percent, depending on such factors as the degree of infestation and the chronicity of the case.

Healing: A few sections were made through channels left by larvae in the submucosa of the esophagus (figs. 5 and 6). Many dead leucocytes, principally disintegrating eosinophils, and fibrin were found in the channels left by the larvae (figs. 5 and 6). Many fibroblasts were found surrounding most of the channels and, unlike their position in the pseudocysts about viable larvae, most of these were observed to be oriented in a direction perpendicular to the healing area (fig. 6). Among these tissues many leucocytes appeared, most of which were viable eosinophils and lymphocytes.

Just what part eosinophils play in the infestation is not well known. Some authors believe that they neutralize toxins given off by internal parasites. At least they are typical components of such infestations. Hadwen (5) showed that they are phagocytic and consume debris and bacteria resulting from invasion of the skin by *Hypoderma*. It is doubtful, however, that this is their principal role in the esophagus, as there was little evidence of phagocytosis. It is known that eosinophils are concerned in the removal of foreign proteins or their split products, and that

they are abundant in conditions of protein hypersensitiveness (6). They probably play a role in neutralization of such foreign proteins as might be elaborated by *Hypoderma* larvae. Bray (3) refers to Bezanlon and Bernard's opinion that eosinophils are cells of the connective tissue rather than of the blood, and that they are often associated with a constructive process, as the building up of tissue. Hydatid cysts and worm infestations were cited as examples. A pseudocyst is sometimes found in infestations of the esophagus by *Hypoderma*. The presence of the eosinophils could hardly be explained entirely on this basis, however, as it is known that a local eosinophilia is rapidly produced when injections of extracts of *Hypoderma* are made. The latter case would seem to call more for neutralization of toxins than for cyst formation. It was observed in certain instances that eosinophils were apparently migrating from the healing area, probably returning to the blood stream.

SUMMARY

1. Some of the principal histopathological reactions resulting from the invasion of the esophageal tissue of cattle by *Hypoderma lineatum* are described and illustrated.
2. Typically the larvae occur in the submucosa and cause inflammatory edema.
3. The edema produced about the larvae distorts the submucous connective tissue and occasionally invades the muscularis mucosa.
4. The infiltration of leucocytes into the pathological area is striking. Most of the hemocytes are eosinophils, lymphocytes, and plasmodocytes. In some cases many of the eosinophils present were myelocytes.
5. Eosinophils may be present at an average density of 476,365 cells per cubic millimeter of tissue.
6. The healing of a larval channel is described and illustrated.

REFERENCES

1. Becker, E. R. and Roudabush, R. L. 1935.—Brief Directions in Histological Technique. 80 pp. Ames, Iowa.
2. Boyd, William. 1934.—A text Book of Pathology. 1047 pp. Philadelphia.
3. Bray, G. W. 1931.—Recent Advances in Allergy. 432 pp. Philadelphia.
4. Hadwen, S. 1918.—Natural Occurrence of Eosinophilias. Jour. Parasitology, 4: 135-137.
5. Hadwen, S. and Fulton, J. S. 1924.—On the Migration of *Hypoderma lineatum* from the Skin to the Gullet. Parasitology 16: 98-106.
6. Taliaferro, W. H. 1929.—The Immunology of Parasitic Infections. 414 pp. New York.

THE DISTRIBUTION OF *PARAGONIMUS**

GEORGE R. LA RUE AND DONALD J. AMEEL

A brief résumé of the results of the study on the distribution of *Paragonimus* was published in the American Journal of Hygiene by one of us (Ameel, 1934). However, it has been deemed advisable to place the complete data at the disposal of those who may be interested in furthering the knowledge of the distribution of this parasite in North America. Though material was secured from many parts of the United States and from several regions in Canada by means of expeditions and purchase, the actual area covered is relatively small.

The genus *Paragonimus* has almost world-wide distribution and for the sake of completeness, the published records are briefly summarized here. Authentic reports for Asia are as follows: Formosa (Manson, 1881; Kubo, 1912), Japan (Baelz, 1880), Korea (Kobayashi, 1918), New Guinea (Cilento and Backhouse, 1927), Indo-China (Salomon and Neveu, 1917), Philippine Islands (Musgrave, 1907), India (Cobbold, 1859; Vevers, 1923), Malay States (Ververs, 1923), Sumatra (Weber, 1891), Java (Noto-Soediro, 1930), China (Maxwell, 1931; Wu, 1935 and 1936). Faust (1929) also lists Siam.

Onorato (1920) published what appears to be an authentic record of a human case of paragonimiasis from Africa. The patient, a resident of Tripoli, had never been further from home than Tunisia. The symptoms described were similar to those of paragonimiasis and the measurements of eggs recovered in the sputum were within the range of *Paragonimus*. After the onset of the disease, the patient suffered from epileptiform attacks which Onorato believed might be due to the presence of worms in the brain. About 7 years after he first began to suffer from the disease, the patient died, but, unfortunately, no autopsy was performed. Onorato and also Salomon and Neveu (1917) casually mentioned a case of paragonimiasis in an Arab reported by R. Wurtz but no reference was given. A copy of the report which was included in an unpublished thesis by M. Crespin presented in 1908 and deposited in the University of Paris was secured through the kindness of Prof. E. Brumpt. The patient, a native of Cayor, had spent 10 years in Senegal and Dahomey in the military service. In addition, he had resided a year on the ivory coast and 5 years in Madagascar. Attacks of hemoptysis began 4 months

*Contribution from the Department of Zoology of the University of Michigan.

This investigation was made with the aid of funds obtained as a Grant-in-aid from the National Research Council and with the aid of a grant from the Faculty Research Funds of the University of Michigan.

previous to the time he was examined by Wurtz who found in the sputum trematode eggs whose lengths were 10 to 12 times the diameter of a red blood corpuscle (0.075 to 0.090 mm.). This places them within the range of the measurements of the eggs of *Paragonimus*. No operculum was observed. Permanent preparations for study consisted of sputum dried upon the slides. Although Professor Brumpt knew of this thesis, he did not include the report in his *Précis de Parasitologie*.

Cawston (1923) while working in Natal found some styled cerariae which he believed indicated the possible presence of *Paragonimus*. He gave no data on their structure, consequently the report cannot be considered as authentic.

Valid records of *Paragonimus* in South America are from Matto Grosso, Brazil (Diesing, 1850), from Peru (Barton, 1910) and from the valley of the Caracas, Venezuela (Iturbe and González, 1919). Khaw (1930) listed a record from Yucatan by Lara (1913) but cited no publication, and no reference to this paper could be found.

Vigueras (1936) cited a statement by Recio (1928) to the effect that *Paragonimus* had been observed in Cuba. Recio's publication could not be obtained.

North American records following that of Ward (1894) are: Kellcott (1894) in a dog at Columbus, Ohio; Abend (1910) in a German miller who had resided in Texas, Colorado and St. Louis and, probably, also in contiguous territory; Nickerson (1911) in a cat at Minneapolis, Minnesota; Hirsch (Hanson, 1911) in 3 cats at Wauwatosa, Wisconsin; Smith (1911) in a wild cat from South Carolina; Hall (1925) in a goat probably from Brooksville, Mississippi; Henry (1927) in 2 minks from Minnesota; Feldman and Essex (1929) in a cat at Rochester, Minnesota; Riley (Wallace, 1931) in a dog from Ithaca, New York, and a cat from Minneapolis, Minnesota; Wallace (1931) in minks and crayfishes in Minnesota; Ameel (1931) in a cat near Ann Arbor, Michigan, in minks and crayfishes from the lower peninsula, and in muskrats from Muskegon and Manistee, Michigan (Ameel, 1932). Null (1910) incidentally mentioned the presence of *Paragonimus* in cats and dogs in the Oriental quarter in San Francisco. No reports regarding the presence of the lung fluke in California have been made since that date and it is suggested that the infected animals may have been brought from the Orient. Prof. Ned Dearborn, formerly of the School of Forestry and Conservation, University of Michigan, found *Paragonimus* in a mink from Wauzeke, Wisconsin, in 1918 and in two minks kept in confinement at Campbellville, Kentucky (Ameel, 1934). The specimens were identified by Dr. B. H. Ransom. One of us (La Rue) has in his collection specimens from a cat from Hillsdale, Michigan (Ameel, 1934).

Examination of crayfishes for the metacercariae of *Paragonimus* was found to be more practical than the examination of the definitive host to determine the presence and distribution of this parasite. Crayfishes are generally numerous and are much easier to collect than the mammalian hosts. These metacercariae are different from other metacercariae, such as *Crepidostomum*, found in the cardiac region of the crayfish, and are easily identified either alive or preserved. Their localization in the heart and adjacent blood vessels makes examination of crayfishes for their presence easy and rapid. In living crayfishes, the metacercariae are recognizable to the naked eye because their white excretory bladders present a good contrast to the yellowish host tissue. The hearts of preserved crayfishes were removed and cleared in liquid phenol to reveal the presence of cysts.

Crayfishes were secured by purchase from Alabama, Arkansas, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Mexico, Michigan, Missouri, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Ontario (Canada), Oregon, Pennsylvania, South Carolina, Tennessee, Virginia, West Virginia and Wisconsin. Subsequently, a large number of crayfishes were examined in South Dakota. The carcasses of fur-bearing animals from Michigan and northern Ohio were also examined.

Infected crayfishes were secured from the following places in Michigan: Ann Arbor, Cass City, Delhi Mills, Dexter, Dixboro, Fenton, Flint, Grand Ledge, Grand Rapids, Lansing, Lowell, Mt. Pleasant, Muskegon, Newport, Owosso, Rockford, Romeo, Saline and Whitehall. Infected crayfishes were found also in the following states: Illinois (Penfield), Indiana (Bluffton, Knightstown, Roanoke), Iowa (Dyersville), Louisiana (Oberlin), Mississippi (Bovina, Macon), Missouri (Galloway, Van Buren), Ohio (Fremont, Jackson), Pennsylvania (Meadville), Virginia (Tip Top), West Virginia (Camp Creek), Wisconsin (Ableman, Burnett, Glen Flora, Holcombe, Marathon City, Menomonie, Pittsville, Waupun, and West Platteville). The following records of infected crayfishes from Minnesota were supplied through the kindness of Dr. F. G. Wallace, formerly of the University of Minnesota: Anoka, Rice Creek—north of Minneapolis, Carver, Chaska, Coon Creek and North Branch. Records for Ontario, Canada, are as follows: Lambeth, Birr and Kippin. In a letter to the junior author, Prof. Thomas W. Cameron, of McGill University, Montreal, Canada, states, "We are finding the condition rather common in Canada, especially northern Canada, in dogs as well as in mink."

In Michigan, the junior author found infected minks from Adrian, Allen, Ann Arbor, Belleville, Brethren, Coldwater, Dekkerville, Diamonddale, Durand, Eaton Rapids, Fennville, Fenton, Flushing, Grand

Rapids, Jones, Jonesville, Manistee, Mikado, Milan, Muskegon, Oakley, Onsted, Paris, Prescott, Quimby, Romeo, Saginaw, Scottville, Twin Lakes, Union City, Vassar, and Whitehall. Infected minks were also secured from Wauseon and Findlay, Ohio. These data enlarge to a considerable extent the areas where *Paragonimus* was known to be endemic.

Though numerous hosts, both crayfishes and minks, from the northern portions of Michigan and Wisconsin were examined no infected animals were found. Wallace, likewise, found no *Paragonimus* metacercariae in crayfishes from the northern part of Minnesota (unpublished data). His collections from this region were principally from Itasca and St. Louis counties. Possibly certain undetermined environmental conditions preclude the maintenance of the infection in these northern regions through the probable total exclusion of the snail host. The snail is evidently the most important factor in limiting the distribution of *Paragonimus*. The mink, unquestionably the normal definitive host in North America, is an inhabitant of all the wooded habitats on the continent where there is permanent fresh water. It is absent only in northern Labrador, in Newfoundland and in Greenland. However, intensive trapping for its valuable pelt has so reduced the numbers of the mink that probably it has disappeared from many portions of its former range, with a resultant diminution of the range of *Paragonimus*. Furthermore, *Paragonimus* through the great reduction in numbers of its definitive host may be so rare in many localities as to escape detection. The crayfish, while far more limited in distribution than the mink, is present in a large part of the same range wherever compatible conditions exist. As will be noted from the list of locality records given below, the snail, *Pomatiopsis lapidaria*, is still unknown from a large part of the region occupied by the mink and the crayfish. Apparently it is uncommon in many parts of its range, though it may be abundant in restricted areas.

While the junior author in his expeditions made hurried visits to most of the eastern and midwestern states, he found no specimens of *Pomatiopsis*. Dr. F. C. Baker, of the University of Illinois, informed him that he had made similar observations on the infrequent occurrence of *P. lapidaria*. In one instance, he and two other experienced collectors traveled 10,000 miles in Illinois and encountered the species but twice. He considers *P. lapidaria* to be a dying species which was abundant during the Pleistocene, becoming rarer in the present fauna. One of us (Ameel) found this snail to be plentiful in local areas near Ann Arbor, Newport and Muskegon, Michigan, and at Belle River, Ontario, Canada.

Numerous records of collections of *P. lapidaria* were obtained through the courtesy of the U. S. National Museum, the Academy of Natural Sciences of Philadelphia, the University of Illinois Museum of

Natural History, and the Museum of Zoology, University of Michigan. These records cover the known range of this species and are as follows: Alabama (Stevenson), Woodville, Wetumpka, Evergreen, Curley, Bollingee), Arkansas (Rogers), Connecticut, Georgia (northern portion), Illinois (La Salle Co., Canton, New Harmony, Milan, Rock Island, Quincy, Joliet), Indiana (New Harmony, Danville, Grand Chain, Lawrenceburg), Iowa (Burlington, Eldora, Iowa City), Kentucky (Bowling Green, Harlan Co., Pine Mountain), Michigan (Ann Arbor, Grand Rapids, Macomb Co.), Minnesota (Fort Snelling, Goodhue, Hastings, Minneapolis), Missouri (Bird's Point, New Harmony, St. Louis), New Jersey (Burlington, Newbold, Red Bank, Riverton, Westville), New York (Niagara Falls, Poughkeepsie), North Carolina (Paint Rock, Roan Mt., Welch Bald), Ohio (Elyria, Columbus, Cincinnati, Red Bank), Ontario, Canada (Brantford, Hamilton, Kingsville), Pennsylvania (Chester Co., Philadelphia), South Dakota (Deuel Co.), Tennessee (Byrdstown, Dove, Cade's Cave, Knoxville, Marion Co.), Texas (Baylor Co., Kendall Co.), Virginia (Lee Co., Luray), Washington, D. C., and Wisconsin (Milwaukee). Other species of the genus in the University of Illinois Museum of Natural History are as follows: *P. cincinnatiensis* (Lea) (Aetna, Tennessee), *P. intermedia* Pilsbry (Santa Cruz, California), *P. californica* Pilsbry (San Mateo Co., California), *P. hinkleyi* Pilsbry (Black Falls and Florence, Alabama) and *P. robusta* Walker (Jackson Lake, Wyoming). *P. hinkleyi*, *intermedia* and *californica* are said to be fairly common, even abundant, in certain localities.

Apparently *Pomatiopsis* is confined to the United States and Canada for there are no existing records of this genus south of the United States. There appears to be no question of the existence of *Paragonimus* in South America. Evidently in the absence of *Pomatiopsis*, some other snail is utilized as a host in the regions where the parasite is present. It is possible that South American members of the *Amnicolidae*, which is closely related to the *Pomatiopsidae*, may be found serving as first intermediate hosts on that continent.

It is interesting to note that there are no records of *Pomatiopsis* from Louisiana, Mississippi and West Virginia, states in which infected crayfishes were found. Though both crayfish and snail hosts appear to be fairly abundant in Kentucky and Tennessee, the junior author failed to find *Paragonimus* in a large number of crayfishes from these states. There is strong probability that *Paragonimus* may be present there as well as in many other states from which, to date, there are no reports of its presence. Conditions are apparently ideal for maintenance of the life cycle in many regions and it is hoped that future investigations will add considerably to the existing knowledge of the distribution of *Paragonimus* on this continent.

BIBLIOGRAPHY

- Abend, L. 1910.—Ueber Haemoptysis parasitaria. *Deutsches Arch. f. Klin. Med.*, 100: 501-511.
- Ameel, D. J. 1931.—More data on the lung fluke, *Paragonimus*, in North America. *Science*, n. s., 74: 493-494.
- 1932.—The muskrat, a new host of *Paragonimus*. *Science*, n. s., 75: 382.
- 1934.—*Paragonimus*, its life history and distribution in North America and its taxonomy (*Trematoda: Troglotremitidae*). *Amer. Jour. of Hyg.*, 19: 279-317.
- Baelz, E. 1880.—Ueber parasitäre Hämoptoë (Gregarinosis pulmonum). *Centralbl. med. Wiss.*, 18: 721-722.
- Barton, A. L. 1910.—Un caso de Distomatosis Pulmonar contraída en el Perú. *La Crónica Médica*, 27: 142-144.
- Cawston, F. G. 1923.—Possible presence of paragonimiasis amongst Indians in Natal. *Jour. Trop. Med.*, London, 26: 263-264.
- Cilento, R. W. and T. C. Backhouse. 1927.—Paragonimiasis: its first recorded occurrence in the territory of New Guinea. *Med. Jour. Aust.*, 14: 79-81.
- Cobbold, T. S. 1859.—On some new forms of entozoa. *Trans. Linn. Soc. London*, 22: 363-366.
- Diesing, K. M. 1850.—*Systema Helminthum*. Vindobonae. Vol. 1, 679 pp.
- Faust, E. C. 1929.—*Human Helminthology*. Lea & Febiger, Philadelphia. 616 pp.
- Feldman, W. and H. Essex. 1929.—Distomatose pulmonaire chez le chat. *Ann. de Par.*, 7: 204-208.
- Hall, M. C. 1925.—The goat as a host of *Paragonimus*. *Jour. Parasitol.*, 11: 227-228.
- Hanson, H. 1911.—*Distoma pulmonale* in Wisconsin. *Johns Hopkins Hosp. Bull.*, 22: 112-114.
- Henry, A. 1927.—Distomatose pulmonaire chez le vison. *Bull. de la Soc. Cent. de Méd. Vét.*, 80: 84-88. (*Red. Méd. Véter. Paris*, 103: 84-88).
- Iturbe, J. and E. González. 1919.—Quelques observations sur les cercaires de la vallée de Caracas. *Laboratoire Iturbe, Caracas*. Pages 1-20.
- Kellicott, D. S. 1894.—Certain entozoa of the dog and sheep. *Trans. Ohio St. Med. Soc.* Pages 122-130.
- Khaw, O. K. 1930.—Remarks on the species of *Paragonimus*. With special reference of the questions of their identity and distribution. *Nat. Med. Jour. of China*, 16: 93-102.
- Kobayashi, H. 1918.—Studies on the lung-fluke in Korea. I. On the life-history and morphology of the lung-fluke. *Mitt. a. d. Med. Hochsch. z. Keijo*, 2: 97-115.
- Kubo, N. 1912.—Morphologie des *Distomum pulmonale*. *Centralbl. f. Bakt. Parasitenk. Originale*, 65: 115-138.
- Manson, P. 1881.—*Distoma ringeri* and parasitical haemoptysis. *China Imperial Maritime Customs. Medical Reports*, 22: 55-62.
- Maxwell, J. L. 1931.—Paragonimiasis in China. A preliminary report. *China Med. Jour.*, Shanghai. 45(1): 43-49.
- Musgrave, W. E. 1907.—Paragonimiasis in the Philippine Islands. *Philipp. Jour. Sci.*, B., 2: 15-65.
- Nickerson, W. S. 1911.—*Paragonimus* in a cat in Minneapolis. *Science*, n. s., 33: 271.
- Noto-Soediro, R. 1930.—*Paragonimus westermani* (Kerbert) bij een kat. *Ned. Ind. Blad. Diergeneesk. Buitenzorg*, 42: 278-280.
- Null, M. M. 1910.—Tochil or endemic hemoptysis. *Northwest Medicine*, 2: 364-366.
- Onorato, R. 1920.—La Paragonimiasi in Tripolitania. *Arch. ital. di scienze med. coloniali*. Tripoli, 1: 1-13.

- Recio, Nodarse y Chediak. 1928.—Manual de Microscopia y Quimica Clinica. T. II. Habana.
- Salomon, M. and R. Neveu. 1917.—Hémoptyxies parasitaires chez les soldats indigènes d'Extreme-Orient. *Le Paragonimus westermani*. Bull. et Mém. Soc. Méd. Hôp. d. Paris, 41: 388-394.
- Smith, A. J. 1911.—Ova of *Schistosomum japonicum*; ova of *S. mansoni*; ova of *Paragonimus westermani*. Proc. Path. Soc. Phila., n. s., 14: 64.
- Vevers, G. M. 1923.—Observations on the genus *Paragonimus* Braun with a re-description of *P. compactus* (Cobbold, 1859), 1899. Jour. Hel. London, 1: 9-20.
- Vigueras, I. P. 1935.—Notas sobre la fauna parasitologica de Cuba (Parte I: Vermes). Mem. de la Soc. Cub. de Hist. Nat., 9: 45-49.
- Wallace, F. G. 1931.—Lung flukes of the genus *Paragonimus* in American mink. Jour. Amer. Vet. Med. Ass., 31: 225-234.
- Ward, H. B. 1894.—Ueber das Vorkommen von *Distoma westermani* in den Vereinigten Staaten. Cent. f. Bakt. u. Parasitenk., 15: 362-364.
- Weber, M. 1891.—De Heer Weber vertoont *Dist. westermani* Kerbert uit de long van een Koningstijger van Delhi (Sumatra). Tijdschrift der Nederl. Dierk. Vereen. 2 serie, III, pp. 83-84.
- Wu, K. 1935.—Notes on certain larval stages of the lungfluke, *Paragonimus* in China. Chinese Med. Jour., 49: 741-746.
- 1936.—Distribution of paragonimiasis in China. I. Chekiang Province. Chinese Med. Jour., Sup. I: 442-448.

A FURTHER CONTRIBUTION TO THE LIFE HISTORY OF
ALLOGLOSSIDIUM CORTI (LAMONT), WITH ESPE-
CIAL REFERENCE TO DRAGONFLY NAIADS AS
SECOND INTERMEDIATE HOSTS*

WILEY W. CRAWFORD

I. INTRODUCTION

Previously two investigators have reported on the life history of *Alloglossidium corti*, a trematode found in the intestine of certain catfish. McCoy (1928) established its life cycle experimentally. He discovered that the cercariae which were found in *Heliosoma trivolvia* penetrated and encysted in both crayfish and dragonfly naiads. When these infested animals were fed to the bullhead, *Amieurus nebulosus*, the adult flukes were recovered. He found the naturally infested metacercariae in crayfish, but not in dragonfly naiads. McCoy believed that he was working with a new species, and thus described it as *Plagiorchis amieurensis*. The taxonomic studies of Simer (1929), Mueller (1930) and Van Cleave and Mueller (1934), however, show that he was dealing with *Alloglossidium corti* instead.

McMullen (1935), in a brief account, confirmed and extended McCoy's results. He reported *Heliosoma campanulata* as an additional snail host and mayfly naiads as another transfer host. He also suggested that small arthropods may serve as second intermediate hosts since very small catfish (40 mm. or less in length) were found to contain the trematode. Both of these workers failed to identify specifically any of the second intermediate hosts concerned.

On September 24, 1933 the author, who is interested in the Odonata as transmitters of trematodes, found a cercaria in some *Heliosoma trivolvis*, collected at Glenwood Lake in the vicinity of Minneapolis, Minnesota, that encysted in the laboratory in a number of species of dragonfly naiads. Subsequent study revealed that when the experimentally infested naiads were fed to the tadpole cat, *Schilbeodes gyrinus* (Mitchell), the metacercaria developed into an adult trematode that proved to be *Alloglossidium corti*. Since the identity of the fluke remained in doubt until the experiments were completed, detailed observations were made and recorded. While these observations confirm what

* Contribution from the Zoology Department of the University of Minnesota which forms a portion of the investigations completed by the author for his Ph. D. Thesis. The writer wishes to express his appreciation to Dr. W. A. Riley, under whose direction this work was done and to acknowledge his indebtedness to Dr. E. M. Walker of the University of Toronto for his assistance in classifying the dragonfly naiads.

has been reported, they also add considerable that is new, especially concerning that portion of the life cycle involving the dragonfly naiad as the second intermediate host. The morphology and development of this stage, as well as the experimental work to specifically identify the dragonfly hosts, are discussed in this paper. The results of the feeding experiments involving *S. gyrinas* as a definitive host are also included since this marks the first time that the adult trematodes have been experimentally reared in this species.

II. THE LIFE HISTORY STAGES

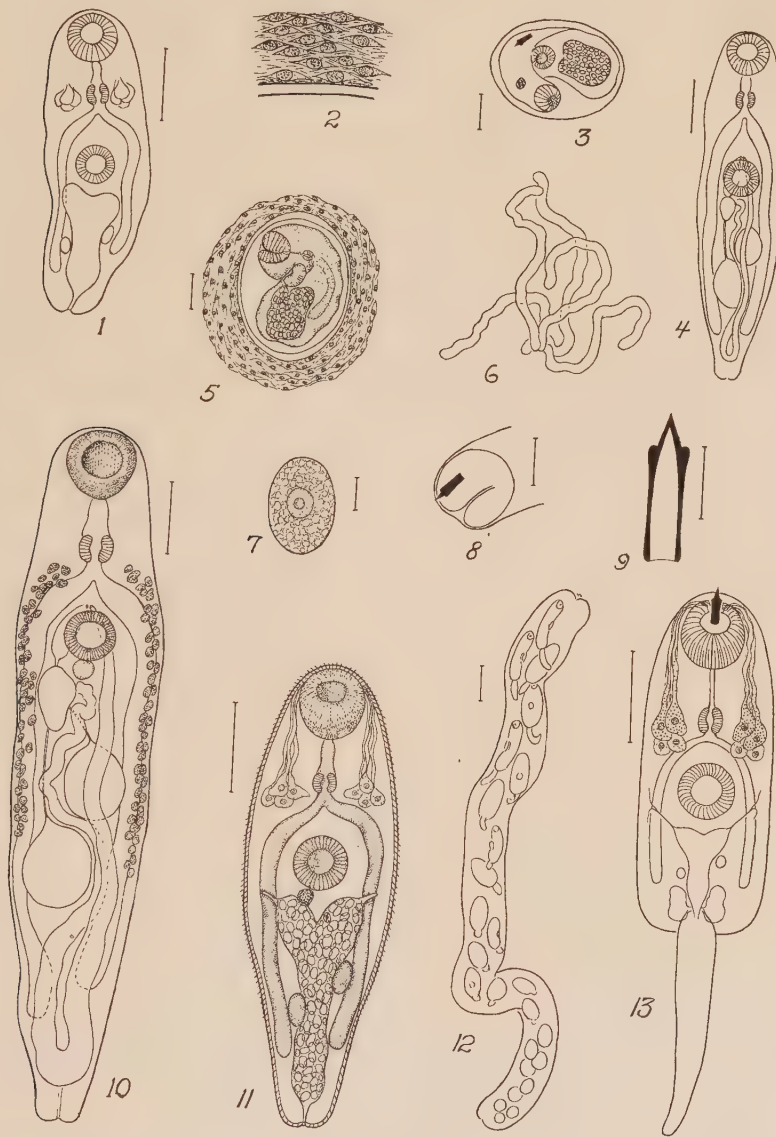
A. Adult. The adult (Plate I, Fig. 10) has been adequately described by McCoy (1928) and other workers. The average measurements for six sexually mature specimens, killed and fixed in hot corrosive-acetic killing fluid, and stained with Ehrlich's haematoxylin, are as follows: body 0.87 mm. by 0.16 mm.; oral sucker 0.08 mm. in diameter; ventral sucker 0.06 mm. in diameter; prepharynx 0.05 mm. in length; pharynx 0.03 mm. in length by 0.03 mm. in width; intestinal caeca 0.020 mm. in diameter; ovary 0.08 mm. by 0.06 mm.; anterior testis 0.09 mm. by 0.08 mm.; posterior testis 0.11 mm. by 0.08 mm.

B. Sporocyst. The sporocysts of *Alloglossidium corti* (Plate I, Figs. 6 and 12) appear as a mass of much elongated, cylindrical sacs twisted around one another. They are not branched, but it is very difficult to trace the individual sacs without rupturing them. However, when this can be done, they are found to be about uniform in caliber and of approximately equal lengths. One typical representative measured 1.8 mm. in length and 0.16 mm. in width. The walls of the sporocysts are very thin and lined with squamous epithelium. The germinal tissue is evidently localized at the proximal end (end opposite the potential mouth) as the germ balls are confined to this portion of the cavity. From the germ balls to the distal end cercariae in all stages of development are present.

PLATE I

- Fig. 1. Metacercaria of *Alloglossidium corti*, ventral view, four days old. Drawn from living specimen. Scale 50 μ .
Fig. 2. Portion of host cyst surrounding cyst of *A. corti*.
Fig. 3. Cyst of *A. corti* seven days old. Scale 35 μ .
Fig. 4. Young *A. corti*, ventral view, six to eight days old, from intestine of catfish. Drawn from permanently mounted specimen. Scale 50 μ .
Fig. 5. Optical section of encysted metacercaria of *A. corti* showing host cyst. Scale 45 μ .
Fig. 6. Sporocysts of *A. corti*.
Fig. 7. Egg of *A. corti*. Scale 10 μ .
Fig. 8. Lateral view of stylet and oral sucker of the cercaria of *A. corti*. Scale 20 μ .
Fig. 9. Stylet of *A. corti*. Scale 10 μ .

- Fig. 10. Adult of *A. corti*, ventral view. Drawn from permanently mounted specimen. Scale 100 μ .
 Fig. 11. Metacercaria of *A. corti*, ventral view, eight weeks old. Scale 50 μ .
 Fig. 12. Sporocyst of *A. corti* showing developing cercariae and germ balls. Scale 100 μ .
 Fig. 13. Cercaria of *A. corti*. Scale 50 μ .



C. Cercaria. With the exception of the rudiments of the testes which are plainly visible in well stained specimens, McCoy has given a good description of the cercaria (Plate I, Fig. 13). The average measurements of six specimens killed in hot 10 per cent. formalin is as follows: body $187.5\ \mu$ in length by $73.8\ \mu$ in width; tail $119\ \mu$ in length; oral sucker $38.5\ \mu$ in longitudinal diameter, $35.4\ \mu$ in transverse diameter; stylet (Plate I, Fig. 28) $19.5\ \mu$ in length by $4.5\ \mu$ in width; ventral sucker $3.9\ \mu$ in diameter; prepharynx $17.2\ \mu$ in length; pharynx $16.9\ \mu$ in width. The testes, in one specimen only, were ovoid and measured $7.6\ \mu$ in length. The left (front) testis was $39\ \mu$ from the posterior end, the right (hind) $30.4\ \mu$.

D. Metacercaria. The description of the metacercarial stage of *A. corti* that follows is based entirely on a study of the living material. The minimum and maximum measurements for six specimens are as follows: body $213\ \mu$ to $267\ \mu$ in length and $95\ \mu$ to $126\ \mu$ in width; oral sucker $48\ \mu$ to $54.6\ \mu$ in diameter; ventral sucker $40\ \mu$ to $45.6\ \mu$ in diameter; pharynx $19\ \mu$ to $23.5\ \mu$ in length; testes $19\ \mu$ to $24\ \mu$ in length and $15.2\ \mu$ to $19.5\ \mu$ in width; crura of intestines $15\ \mu$ to $20\ \mu$ in diameter.

The metacercaria of *A. corti* (Plate I, Figs. 1, 11) is small and moderately active. The body is slipper-shaped, the anterior end forming the blunt end, and the posterior end the tapering one. Small cuticular spines are present over the entire body surface, but are less conspicuous on the posterior end. The oral sucker is subterminal, and has a large circular opening. The ventral sucker is located slightly anterior to the middle of the body. The ratio of the size of the oral sucker to that of the ventral sucker is 5:4.

The digestive system is well developed. The prepharynx is wide and extensible, its length when fully extended exceeding that of the pharynx. The pharynx is well defined. The esophagus is very short and somewhat narrower than the prepharynx. The intestinal branches diverge sharply from their point of origin, and extend nearly to the posterior end of the body. They are slightly inflated.

Of the reproductive complex only the rudiments of the testes and the ovary are present. The testes are obliquely placed, located inside the intestinal branches and lateral to the excretory bladder. They are equal in size, elliptical and compact. While the size of the testes is rather uniform and averages $21.7\ \mu$ by $17.5\ \mu$, a few cases have been observed where the length was as great as $30\ \mu$. From three to five germ cells are present in each testis. The ovary is situated slightly to the right of the median line and posterior to the ventral sucker.

The excretory bladder is inconspicuous and Y-shaped, reaching only to the region of the ventral sucker. The stem portion is several times longer than the vesicular-shaped cornua. The excretory granules are exceptionally large, and average from $9.5\ \mu$ to $12\ \mu$ in diameter.

The stylet glands described for the cercaria are still present, yellowish green in color and quite conspicuous. It is possible that since they persist, they have taken over a new task and function as modified stylet glands in the metacercaria.

III. OBSERVATIONS ON THE BIOLOGY OF THE CERCARIA

A. *General behavior.* The cercariae exhibit a marked periodicity in swarming, as they leave the snail host only between 7:00 and 9:00 P. M. For a brief period after escaping they drift passively through the water, attached to long delicate strands of a mucous-like substance emitted from the snail. Then they settle to the bottom where they crawl over the substrate with the aid of their suckers in typical measuring worm fashion. At frequent intervals the crawling is interrupted while they swim vertically toward the surface for a short distance before sinking to the bottom again. This latter behavior undoubtedly increases their chances of entering the dragonfly nymph as it places them in position to be drawn into the respiratory current. The cercariae do not respond to light.

In a series of observations to determine the longevity of the cercariae, it was found that they would live for over 48 hours in tap water, and from 60–72 hours in pond water. Those subjected to the pond water were able to swim free of the substrate on the second day, but not on the third. If, however, the water was agitated and the cercariae shaken free, they would drift for several minutes before finally sinking to the bottom again. This observation indicates that the dragonfly naiads are subjected to infestation from a cercaria for almost three days after the latter enters the water. Whether or not, however, a cercaria has the vitality to penetrate the wall of the rectum and encyst during the later hours of its life is a matter for consideration in attempting to determine how long a given cercaria is infective.

B. *Infestation in snails.* Observations have been made on *Heliosoma trivolvis* collected from Glenwood Lake near Minneapolis, Minnesota, during the summer and early fall of 1933 and the spring of 1934 and from Lake Johanna in the vicinity of St. Paul, Minnesota, in the spring of 1934. No mature cercariae were discovered at Glenwood Lake until September 24, although some immature ones were found in three snails collected on September 15. On September 24, of the 151 snails collected, 80 of which were but half-grown, 12 snails were infested with the cercariae of *A. corti*, 9 larger snails and 3 smaller ones. On April 11, 1934, two of the 23 snails collected at Glenwood Lake contained cercariae, but on May 23 none were found in a collection of 45. On May 16, 1934, 167 snails were collected at Lake Johanna, 3 of which were infested, but no cercariae were found in 126 snails collected the last week of the month.

The evidence derived from these results show that: (1) the cercariae reach maturity in the early fall; (2) the snails remain infested throughout the winter and spring; (3) the percentage of infestation in the snails is small. Additional evidence that the snails retain their infestation for a long time is furnished by an observation made on one of the small infested snails collected from Glenwood Lake on September 24, 1933. Cercariae continued to escape from this snail until June 29, 1924, a period of nine months. Two weeks later it was killed and examined for sporocysts, but none was found.

IV. THE DEVELOPMENT OF THE METACERCARIA IN DRAGONFLY NAIADS

A. *Entrance and encystment of cercaria*: The cercariae gain entrance to the body of the dragonfly naiad by being drawn in with the respiratory current into the rectum, after which they penetrate the wall of this organ. In the course of 30 minutes as many as 14 cercariae have been observed to pass into the anal opening, during which time none was expelled. While the cercariae crawl all over the naiads, none has even been seen piercing the exoskeleton.

When the cercaria penetrates the wall of the rectum, the tail becomes detached, remaining lodged in the tissue where it dies, becomes infiltrated with a brown pigment and hardens. Within the body, the tailless cercariae encyst ordinarily among the muscle fibers of the abdominal body wall, frequently in the fatty tissue. Occasionally encystment occurs in the rectal gills. No cysts have been found in the thorax. Cercariae have been observed among the muscle fibers within an hour after the naiad has been exposed, indicating that the entire process of entrance and encystment is completed in a comparatively short time.

One very heavily infested naiad of *Gomphus spicatus* showed the following distribution of cysts in the abdomen: first to third segments 3 cysts; fourth segment 12; fifth segment 45; sixth segment 60; seventh segment 85; eighth segment 100; ninth segment 38; tenth segment 9; laminae of gills 7; fat body 40; total number present 404 cysts.

The host cyst does not begin to develop until the third day following encystment. By the sixth day it is well formed and consists of a layer, 26.6 μ to 34.2 μ in thickness, of spindle-shaped cells with large nuclei. (Plate I, Fig. 2).

B. *Increase in size of the cysts in experimentally infested dragonfly naiads*. The newly formed cysts average 116.9 μ in length and 89.1 μ in width. They increase slowly in size during the first seven weeks; then growth proceeds rapidly for the next two weeks, at which time the maximum size is reached. Cysts six months old are approximately the same size as those nine weeks old. The following measurements, which represent an average of the length and width of ten specimens, show the rate

of increase in the size of the cysts: 4 days, 120.7 μ by 95.6 μ ; 1 week, 121.3 μ by 96.2 μ ; 2 weeks, 124.1 μ by 97.5 μ ; 4 weeks, 137.7 μ by 105.3 μ ; 7 weeks, 143.4 μ by 112 μ ; 9 weeks, 178 μ by 156 μ ; 6 months, 178.8 μ by 151.4 μ . The largest cyst measured at the end of nine weeks was 205.2 μ in length and 174.8 μ in width; the smallest, 152 μ in length and 114 μ in width. These cysts were larger than those found in the naturally infested naiads.

When first formed, the cyst wall averages less than 3 μ in thickness. The subsequent growth of the cyst wall proceeds unevenly, the sides of the mature cyst (Plate I, Fig. 5) attaining an average thickness of 7.8 μ ; the ends 4.5 μ .

C. Changes in the developing metacercaria of experimentally infested dragonfly naiads. With few exceptions the development of the metacercaria results from the growth and regulation of the cercarial stage, the rate of which is correlated closely with the increase in the size of the cyst. The measurements for the cercaria and the metacercaria have been given previously. The excretory granules, which are very minute and difficult to see in the cercaria, upon reaching the excretory bladder of the encysted trematode coalesce to form large conspicuous structures. The intestinal branches, present in the cercarial stage as solid cords of cells, hollow out during the first week of encystment. The fundament of the ovary was noted for the first time the seventh week of development, but since its position is obscured by the right cornu of the excretory bladder, it is possible that it may have appeared sooner. Although the stylet may persist until the ninth week following encystment, its presence is rare after the fourth week. It does not remain in position after the first day, but lies wedged in between the metacercaria and the cyst wall (Plate I, Fig. 3).

V. EXPERIMENTS AND OBSERVATIONS TO IDENTIFY THE NAIAD HOSTS

The results which identify certain dragonfly naiads as second intermediate hosts of *A. corti* are derived from several experiments performed in the fall of 1933. The naiads were collected from Lake Johanna while still very young during the month of August, and brought to the laboratory where they were placed in two-gallon white porcelain pans equipped as aquaria with a layer of cleaned sand in the bottom, Minneapolis city water and sprigs of Elodea and Ceratophyllum for the naiads to cling to. They were fed cyclops cultured in large quantities for this purpose. As many as 40 or 50 naiads could thus be kept in a single aquarium for several weeks with only a small amount of cannibalism occurring. They were free of infestation as repeated examinations of dozens of their number failed to disclose the presence of cysts. However, after the

naiads were allowed to become infested they were segregated in lots of from 3 to 5 in standard butter dishes equipped as small aquaria, and cared for as before.

After a number of preliminary trials had shown that the cercariae from *H. trivoltis* would encyst in a number of different species of dragonfly naiads the final critical experiments were conducted, with the following results: (1) On October 2, eight naiads of *Leucorrhinia intacta* were exposed to cercariae. One of the naiads died on the third day. A *post-mortem* examination revealed the presence of over 100 cysts. The remaining naiads, examined at successive intervals of 5 days, were infested, the number of cysts per host ranging from 50 to 125. (2) On October 4, eight naiads of *Tetragoneuria cynosura* were subjected to cercariae. One of the naiads was killed by the others. The other seven, examined at successive intervals of 5 to 6 days, were infested, the smallest number present per naiad being 40, the largest 160. (3) On October 4, eight naiads of *T. spinigera* were also placed with cercariae. One died on the third and another on the twelfth day following infestation. Both were infected, one with 27 cysts, the other with 75. The rest, examined as usual at intervals of 5 to 6 days, disclosed the presence of cysts in numbers ranging from 36 to 125 per naiad. (4) On October 6, six naiads of *Gomphus spicatus* were exposed to cercariae. Subsequent examination at intervals of 5 to 6 days revealed that each of the naiads carried a heavy infestation—from 115 to 205 cysts.

Control specimens for each of the experiments described above were isolated in butter dishes, and subjected to the same conditions as the infested naiads. Upon examination every one proved to be negative. In addition to the controlled experiments 250 *G. spicatus* naiads were infested for the purpose of studying the development of the metacercariae and for feeding to the primary host. Every one of this number when later examined was found to be infested.

In the naiads of *L. intacta* and *G. spicatus* the cysts were embedded in the muscles of the abdominal body wall, sometimes in the fatty tissue and occasionally in the gill lamellae. In the *Tetragoneuria* naiads the cysts were equally distributed between the muscles and the fatty tissue, and none was observed in the gills. Both the increase in the size of the cyst and the development of the metacercariae are evidence that these dragonfly naiads are possible intermediate hosts for *A. corti*.

Attempts were made to infest a number of other dragonfly naiads. Those of *Epicordulia princeps* were not susceptible. While the cercariae encysted, occasionally in large numbers, in *Libellula luctuosa* and *Platthemis lydia*, these naiads are impossible hosts because in the case of the former the cysts died, became impregnated with brown pigment and

hardened; in the latter, development appeared to be arrested and the cysts did not increase in size.

In May and June* of 1933 the metacercariae of *A. corti* were found encysted in 27 per cent of the naiads of *L. intacta* collected from Glenwood Lake, in numbers from 5 to 27, and in 21 per cent of the naiads of *L. intacta* from Lake Johanna in numbers from 7 to 35, thereby confirming the experimental results that this dragonfly is the intermediate host of this trematode. Thirty-four per cent of the naiads of *Leucorrhinia* sp. from Lake Johanna also harbored the cysts in numbers from 7 to 14. While the naiads of *T. spinigera*, *T. cynosura* and *G. spicatus* can be experimentally infested in the laboratory, no cysts have been discovered in any of these naiads collected and examined either in the fall or in the spring. The difference in habitat may account for this condition, as the snail *H. trivolvis* and the *Leucorrhinia* naiads are found in the swampy portions of the lake, while the naiads of the *Tetragoneuria* species and *G. spicatus* inhabit the sandy bottoms.

Although the naiads of *Packydiplax longipennis*, *Libellula luctosa* and *Sympetrum semicinctum* were abundant in the same locality at Lake Johanna where the infested *Leucorrhinia* naiads were collected, none was found to contain cysts.

The cysts in the naturally infested *Leucorrhinia* naiads are distributed the same as described for the experimentally infested ones. Usually the cysts are colorless, but occasionally in older infestations the cyst wall becomes infused with a brown pigment. The cysts are oval in shape and very small, ranging from 129 μ to 167 μ in length and from 110 μ to 129 μ in width. The most striking feature about them is the excretory bladder filled with the large opaque granules.

VI. THE EXPERIMENTAL WORK ON SCHILBEODES GYRINAS

The feeding experiments involving the catfish *S. gyrinas* consist of a preliminary and a final experiment, performed six months after the dragonfly hosts had been experimentally determined. Previous to this, several attempts had been made to infest various species of frogs and turtles, but without success.

In the preliminary experiment two specimens of *S. gyrinas* collected from the Minnesota River near Shakopee, Minnesota, were fed approximately 25 cysts of *A. corti* each on March 28, and again on March 31. Three days later 27 young flukes, of two different sizes, were recovered from the small intestine of one catfish and 21 from the other. No adults were present. Except for a slight increase in size, these young trematodes were identical with the metacercariae, showing that they were the ones fed.

* The adults of the genus *Leucorrhinia* emerge throughout the month of June in latitude of Minneapolis.

For the final experiment 16 additional catfish were secured from the same locality, and kept in the laboratory for three weeks before the experiment was started. Then 10 were examined to determine the percentage of natural infestation. Only two were infested; one with three adult *A. corti*, the other with one. Since the natural infestation was so extremely light, it was decided that the remaining six specimens could be used without invalidating the results. From the time that the catfish reached the laboratory until the investigations were concluded they were kept singly in one gallon aquaria, covered over with a wire screen of very fine mesh to prevent their jumping out and to guard against accidental infestation. The cysts of *A. corti* fed were from naiads of *G. spicatus*, experimentally infested the previous fall.

The summary of the feeding experiments and their results follows: No. 1 was fed 20 cysts April 21, 40 more April 27 and 30 more May 3. Upon examination 27 trematodes, represented by three different sizes, were recovered from the small intestine: 7 sexually mature, 12 almost grown, 7 about half grown. No. 2 received 25 cysts April 21, 50 April 27 and 40 May 3. May 8, 34 worms of three different sizes were recovered from the small intestine. None was sexually mature. No. 3 was forcibly fed 30 cysts on April 21. It died the following day. Thirteen young distomes, all the same size, were found in the small intestine. No adults were present. No. 4 received 50 cysts April 21, 50 April 26, 30 May 1 and 40 May 6. Examination on May 13 disclosed 46 trematodes of four different sizes in the small intestine: 12 sexually mature with numerous eggs in the uterus, 9 containing a few eggs in the uterus, 11 about one-half mature and 15 about one-third mature. No. 5 was fed on the same days as No. 4 and approximately the same number of cysts. When examined on May 13, 35 worms distributed among four different sizes were found. No. 6 received 40 cysts on April 21 and 50 more on May 6. Upon examination May 22, 17 mature flukes, each with the uterus filled with eggs, and 21 young flukes were recovered in the small intestine.

Thus 250 trematodes in various stages of development were recovered from the intestine of the eight catfish that were fed cysts from experimentally infested dragonfly nymphs. The graduation in size of the worms, which was occasioned by the cysts being fed at intervals, shows that the metacercariae develop in the intestine of the catfish. The enormous increase in both the percentage of infestation and in the total number of trematodes found as compared with the controls is sufficient evidence that the adults recovered resulted from the cysts fed and not from natural infestation.

The data available indicate that it takes from 17 to 21 days for the metacercariae to reach sexual maturity in the final host.

Three attempts were made to hatch the eggs of *A. corti* in the laboratory, but each was unsuccessful. Therefore, it was impossible to complete the life cycle experimentally by infesting the snail host.

VII. CONCLUSIONS

1. Additional studies regarding the life history of *Alloglossidium corti* have been presented.

2. A number of different species of dragonfly naiads may serve as the second intermediate host. Naiads of *Leucorrhinia intacta* and *Leucorrhinia* sp. were found naturally infested. While naiads of *Tetragoneuria cynosura*, *T. spinigera* and *Gomphus spicatus* could be experimentally infested, none was found to be naturally infested.

3. As many as 404 cysts have been recovered from a single experimentally infested dragonfly naiad. Forty-seven was the largest number found in a naturally infested naiad.

4. The experimental results involving the tadpole cat *Schilbeodes gyrinas* have been presented for the first time. The metacercariae mature in the intestine of this catfish in 17 to 21 days.

5. The percentage of infestation in snails is rather low. The cercariae become mature in late summer, and the snails remain infested throughout the winter.

6. The cercariae escape from the snail between 7:00 and 9:00 o'clock in the evening. They enter the dragonfly naiads passively by being drawn into the rectal respiratory chamber by means of the respiratory current, after which they penetrate the wall of the rectum and encyst in the muscles and fatty tissue of the abdomen.

BIBLIOGRAPHY

- Lamont, Marion E. 1921.—Two new parasitic flatworms. Occ. Papers Mus. Zool. Univ. Mich. No. 93, 6 pp.
- McCoy, O. R. 1928.—Life history studies on the trematodes from Missouri. Jour. Parasit., 14: 207-228.
- McMullen, Donald B. 1935.—The life history and classification of two Allore-
adiid-like Plagiorchids from fish. Jour. Parasit., 21: 369-380.
- Mueller, Justus F. 1930.—Trematode genus Plagiorchis in fishes. Trans. Amer. Micro. Soc., 29: 174-177.
- Simer, P. H. 1929.—Fish trematodes from the lower Tallahachie River. Amer. Midland Nat., 11: 563-588.
- Van Cleave, Harley J. and Justus F. Mueller. 1934.—Biological and ecological survey of the worm parasites of Oneida Lake fishes. Roosevelt Wild Life Annals, 3: 161-334.

A NEW VARIETY OF *PLASMODIUM RELICTUM* FROM THE ROBIN*

CLAY G. HUFF

A new strain of avian malaria, isolated from an American robin in April, 1935, is morphologically identical with known strains of *Plasmodium relictum*, but differs biologically so much from these strains as to be of considerable interest and importance as a laboratory strain. Since this strain is already being used in several series of experiments, it would seem advisable at this time to describe and name the organism. This variety, which is being designated as *Plasmodium relictum* var. *matutinum*, differs from other known strains of this species (a) in possessing a strict quotidian periodicity and a high degree of synchronism of segmentation, (b) in the very acute and short-lived infection produced in canaries, and (c) in its low degree of infectivity for *Culex* mosquitoes. Investigations on other characteristics of this variety are being carried out by the writer and by other investigators in this laboratory as parts of other problems. These include (a) studies on gametocyte formation, (b) cross-immunity experiments with other strains, and (c) temperature reactions of the bird to this parasite.

DESCRIPTION

Plasmodium relictum matutinum, new variety

Hosts. American robin (*Planesticus migratorius*); canary (*Serinus canarius*) (experimental).

Morphology. Young trophozoites: rounded or ameboid, in erythrocytes and erythroblasts, most numerous about two hours following beginning of daylight hours. Pigment not visible in youngest trophozoites. Six-hour old trophozoites: mostly ameboid, cytoplasm vacuolated or not so, pigment not yet visible. Twelve-hour old trophozoites: about the size of the erythrocyte nucleus, beginning to displace the latter; pigment, one or more fine granules; nucleus, round or oval and sharply defined. Eighteen-hour old schizonts: about twice the size of erythrocyte nucleus, which is further displaced; nuclear material of parasite in two or three masses; pigment, many-granules, in a clumped mass; a clear vacuole commonly present near pigment. Mature schizonts: spherical or oval; nuclei in several masses; pigment, a clump of granules. Segmenters with 10 to 22 merozoites: pigment mass, centrally located; average number of merozoites, 14; segmentation highly synchronous; maximum percentage of segmenters appearing after twelve hours of darkness. Gametocytes: indistinguishable from gametocytes of the German, Whitmore, and Virginian strains of *Plasmodium relictum*. Microgametocytes: cytoplasm, very pale blue; nucleus, rounded or irregular, staining a deep

*From the Department of Hygiene and Bacteriology, University of Chicago. This research was aided by a grant for Malaria Field Studies from the International Health Division of the Rockefeller Foundation. Grateful acknowledgment is made to Mr. W. B. Taber, 3rd, for aid in securing the original strain of parasites from a robin. This paper was accepted for publication October 22, 1936.

eosin; pigment, in ten to twenty discrete spherical granules, evenly scattered over the parasites. Macrogametocytes: similar to microgametocytes, except cytoplasm deep azure. Infection in canaries very acute, terminating usually on fourth or fifth day following appearance of parasites in blood. Pathogenicity, slight.

Sporogenous cycle in mosquitoes. One experimental transmission has been effected by *Culex fatigans*; experimental index of infection for *Culex fatigans* and *C. pipiens* 2% or less.

Type locality: Kansas, Illinois.

Type host: Robin (*Planesticus migratorius*).

Derivation of name: *matutinus*, -a, -um; adj. [*L.* = pertaining to the morning.]

DISCUSSION

Previous studies on the length of the asexual cycle of *Plasmodium relictum* by L. G. Taliaferro (1925) have shown it to be approximately 30 hours in the Whitmore strain and by Gingrich (1929) to be about 36 hours in the Virginian strain. Segmentation in these and other similar strains of this species has been known to be lacking in synchronism. One of the most striking things about this new variety, *matutinum*, is its very strict periodicity and high degree of synchronism in segmentation. Figure 1 shows the periodicity of segmentation in three canaries with *matutinum* infections (1R, and 18R) as contrasted with the lack of periodicity in the German strain of *relictum* (402G). It will be noted

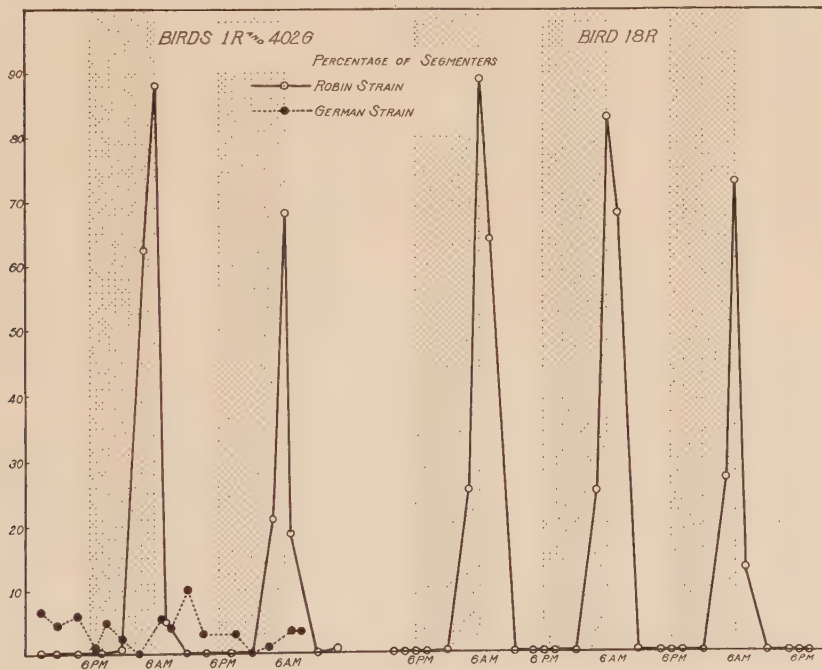


Fig. 1. Periodicity of segmentation in *Plasmodium relictum matutinum* (1R and 18R) and *Plasmodium relictum* (German strain, 402G). The stippled areas indicate periods of darkness.

here that at 6 A. M. the percentage of segmenting forms is usually above 50 and is sometimes as high as 90 in the infections with *matutinum*, whereas it is less than 10 in the German *relictum*. Segmentation begins in *matutinum* infections regularly after six hours of darkness and is usually completed after six hours of light. On a schedule of 12 hours of light and 12 hours darkness the peak of segmentation, therefore, falls at the time when the bird is changed from darkness to light. In conformity with the findings of Boyd (1929) on *P. cathemerium* this period of segmentation has been found, when the bird was subjected to an artificial day and night, to shift in such a way as to fall at the close of the period of artificial night.

It is noteworthy that two varieties of the same species should differ so widely in the periodicity and synchronism of segmentation. It is also interesting to note that this new variety which is not markedly different in many ways from *P. cathemerium* should possess the characteristic of segmenting at a time of day antipodal to that of the latter species, which is known to segment in greatest numbers after 12 hours of light. When these two strains of organisms were inoculated into the same bird there resulted a semidiurnal periodicity, as would be expected if the separate strains retained their characteristic periods of segmentation. (See Figure 2.) It is apparent that the physiological changes in the bird

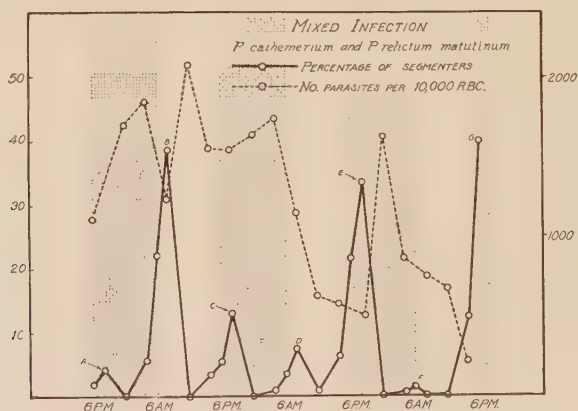


Fig. 2. Mixed infection of *Plasmodium cathemerium* and *Plasmodium relictum matutinum* in the same bird. Stippled areas indicate periods of darkness. Note that the peaks of segmentation occur at 12-hour intervals. The peaks at a, c, e, and g presumably represent the *cathemerium* part of the infection, while the peaks at b, d, and f represent the segmentation of the *matutinum* portion of the infection.

brought about by subjecting it to equal periods of light and dark, affect the two strains of parasites so as to produce antipodal effects on their times of segmentation. It has been shown by Huff (1935) that the peak of segmentation in *P. hexamerium* occurs around 8 to 9 A. M., and by

Huff and Bloom (1935) that in *P. elongatum* this peak occurs between 8 and 10 A. M. However, *P. relictum matutinum* is the first avian *Plasmodium* of the group possessing round gametocytes to be shown to segment chiefly in the morning.

The various strains of *P. relictum* hitherto studied possess a fairly high degree of chronicity. The infection in some individual canaries follows the course characteristic of *P. cathemerium*, i.e., an acute primary infection with the almost complete disappearance of organisms from the blood subsequently. More frequently, however, the infections are of the nature of the "extended irregular" type described by Ben-Harel (1923). These infections may last as long as three weeks. In any of these strains it is much easier to find parasites in the blood during latency than it is in infections with *P. cathemerium*. *P. relictum matutinum* uniformly produces an acute primary infection of short duration (4–5 days or less) followed by a latency during which parasites are as difficult to demonstrate in the blood as they are at the similar stage in *cathemerium* infections.

The infectivity of this new variety for the species of mosquitoes so far tried is strikingly lower than that of previously known strains. In 1930, the writer found an infection rate of 89.4% and 87.3% respectively for the Boston and Whitmore strains of *P. relictum* in experimentally fed *Culex pipiens*. The experimental index of infection for the German strain of *relictum* in *C. pipiens* was found (1934) to be approximately 42%. In 1927, only two specimens of *C. fatigans* (*quinquefasciatus*) fed on birds infected with *P. relictum* (Virginian strain) were dissected and these were infected.

In the study here presented, further feedings of *C. fatigans* were made on infections with the German strain of *P. relictum* and with the

TABLE 1.—Results of dissection of *Culex* mosquitoes fed on *P. relictum* (German strain) and *P. relictum matutinum*

Lot No.*	Strain of parasites	No. dissected	No. positive	Per cent infected
A	<i>matutinum</i>	2	0	1.5
B	"	4	1	
C	"	9	0	
D	"	7	0	
E	"	2	0	
F	"	36	1	
G	"	30	0	
H	"	40	0	
I	"	5	0	
1.5				
J	<i>matutinum</i>	80	2	2.5
K	German	1	1	
L	"	40	23	
M	"	72	49	
73.0				

* All lots consisted of *Culex fatigans* except lot J, which was a mixed culture of *C. pipiens* and *C. territans*.

variety *matutinum*. Feedings were also made upon *matutinum* infections of a mixed lot of *Culex pipiens* and *C. territans* bred from larvae collected in the field. The results, shown in Table 1, indicate that *Culex fatigans* (and probably *territans* and *pipiens*) is very slightly susceptible to the new variety as compared with previously known strains of *P. relictum*. This is true in spite of the fact that gametocytes were very numerous in the blood of the birds upon which the mosquitoes fed. It seems apparent that other species of insects must serve as vectors of this variety.

A statistical comparison was made upon the size of gametocytes and the number of merozoites produced by segmenters in the German strain of *relictum* and the new variety. Outlines of the gametocytes were drawn by *camera lucida* from smears taken at 3-hour intervals for a 24-hour period and these were measured by polar planimeter. Significant differences were not found in gametocyte size or number of merozoites produced by the two strains.

SUMMARY

A new variety of *Plasmodium relictum* isolated from an American Robin is described as variety *matutinum*. It is indistinguishable by the criteria of staining, morphology, and size from the German strain of *P. relictum* and probably also from the Whitmore and Virginian strains, but it differs from all of these strains by every physiological criterion employed.

REFERENCES

- Ben-Harel, S. 1923.—Studies of bird malaria in relation to the mechanism of relapse. *Amer. Jour. Hyg.*, 3: 652-659.
- Boyd, G. H. 1929.—Induced variations in the asexual cycle of *Plasmodium cathemerium*. *Amer. Jour. Hyg.*, 9: 181-187.
- Gingrich, W. 1929.—Periodicity in bird malaria (*Plasmodium inconstans*). *Jour. Paras.*, 16: 98.
- Huff, C. G. 1927.—Studies on the infectivity of Plasmodia of birds for mosquitoes, with special reference to the problem of immunity in the mosquito. *Amer. Jour. Hyg.*, 7: 706-734.
- 1930.—Individual immunity and susceptibility of *Culex pipiens* to various species of bird malaria as studied by means of double infectious feedings. *Amer. Jour. Hyg.*, 12: 424-441.
- 1934.—Comparative studies on susceptible and insusceptible *Culex pipiens* in relation to infections with *Plasmodium cathemerium* and *P. relictum*. *Amer. Jour. Hyg.*, 19: 123-147.
- 1935.—*Plasmodium hexamerium*, n. sp. from the bluebird, inoculable to canaries. *Amer. Jour. Hyg.*, 22: 274-277.
- Huff, C. G. and Bloom, W. 1935.—A malarial parasite infecting all blood and blood-forming cells of birds. *Jour. Inf. Dis.*, 57: 315-336.
- Taliaferro, L. G. 1925.—Infection and resistance in bird malaria with special reference to periodicity and rate of reproduction of the parasites. *Amer. Jour. Hyg.*, 5: 742-789.

A REVISION OF THE GENUS *PODOCOTYLE* (ALLO-
CREADIINAE), WITH A DESCRIPTION OF EIGHT
NEW SPECIES FROM TIDE POOL FISHES
FROM DILLON'S BEACH, CALIFORNIA

JAMES T. PARK

In July of 1933 a number of species of *Podocotyle* Odhner (1905) were collected by me at the temporary Marine Laboratory of the College of the Pacific at Dillon's Beach, California. Study of these trematodes reveals the necessity of revision of their generic characters in respect to the seminal vesicle, which revision is here presented.

I am greatly indebted to Prof. Charles A. Kofoed at the University of California for his helpful suggestions, to George S. Myers of the U. S. National Museum for identification of hosts and to Prof. Harold W. Manter of the University of Nebraska for his loan of identified specimens of *Podocotyle atomon* and *P. olssoni*.

Historical discussion: Since the history of the genus *Podocotyle* has been reviewed by Odhner (1905) and Manter (1926), no attempt is made here to go back further than 1905. When Odhner (1905) created *Podocotyle* for *Fasciola atomon* Rudolphi (1802), he listed three species in it, viz., *P. atomon* (Rudolph, 1802), *P. reflexa* (Creplin, 1825) and *P. olssoni* Odhner (1905). Nicoll (1909) created a subspecies *P. atomon* var. *dispar*, because of the occurrence of an asymmetrical group of vitelline follicles in front of the ventral sucker on the right side instead of being absent as in *P. atomon*. Since then, *P. syngnathi* Nicoll (1913), *P. pennelli* Leiper and Atkinson (1914), *P. levenseni* Isaitschikov (1928), *P. odhneri* Isaitschikov (1928), *P. ayu Takahashi* (1928), *P. pearsei* Manter (1934), and *P. lanceolata* Price (1934) have been added to this genus. The description of *P. pennelli* is incomplete. Although Manter (1926) pointed out the variation in the distribution of vitellaria and the relative constancy in the length of the oesophagus, Isaitschikov (1928) later attempted to classify the species of the genus in the main according to the distribution of vitellaria and the direction of the lobes of the ovary. However, this basis for the classification of the species of *Podocotyle* faces too many difficulties, since the distribution of the vitellaria varies in a wide range within the species, as pointed out by Manter (1926) and noted in my present study. Another basis for specific distinctions must be sought. The following characters are less variable: (1) body dorsoventrally flattened or cylindrical; (2) seminal vesicle straight, sinuous or coiled; (3) extension of cirrus sac anterior or posterior to acetabulum; (4) morphological structures of genital organs; (5)

relative position of testes in relation to each other, even with allowance for age changes.

Podocotyle (Duj.) Odhner (1905)

Syn. *Sinistroporus* Stafford (1904)

Podocotyloides Yamaguti (1934)

Generic description: *Allocreadiinae*; body elongated or elliptical in outline, dorso-ventrally flattened or cylindrical; cuticula smooth or ridged; ventral sucker larger than oral sucker; oesophagus about twice as long as the pharynx at the most; excretory pore terminal or subterminal; excretory bladder elongated or clavate, extending anteriorly to the level of the middle of the posterior testis or beyond to the posterior edge of acetabulum; genital pore pre-acetabular, at left of body; cirrus sac elongated or clavate, extending to the anterior edge of or posterior to acetabulum; its anterior part protruded, protrusible, or not (?) protrusible; seminal vesicle straight, sinuous or coiled within cirrus sac; testes post-medial, smooth or lobed; ovary pre-testicular, smooth or lobed; seminal receptacle large; Laurer's canal present; coils of uterus intercaecal, between acetabulum and ovary; eggs without polar filament; vitellaria lateral, largely outercaecal, extending from the region of acetabulum to posterior end of body. Parasites of marine fishes.

Type specimen: *P. atomon* (Rudolphi, 1802).

The study of the species of *Podocotyle* here described is made from specimens flattened under cover slip or slide.

Podocotyle endophrysi sp. nov.

Plate I, figs. 5, 6, 8 and 9

Description: Body elongated, dorso-ventrally flattened, tapering anteriorly from acetabulum and bluntly pointed posteriorly, 1.47–3.41 (2.43) mm. in length by 0.39–0.80 (0.58) mm. in width at level of acetabulum; cuticula very thin; oral sucker terminal, 0.13–0.24 (0.17) mm. in length by 0.13–0.24 (0.16) mm. in width; ventral sucker embedded or slightly pedunculated, at 0.22–0.32 (0.27) total length from anterior end, about 1.6 times larger than oral sucker, 0.17–0.30 (0.23) mm. in length by 0.21–0.33 (0.28) mm. in width; prepharynx 0.03 mm. in maximum length; pharynx 0.08–0.13 (0.10) mm. in diameter; oesophagus slender, sometimes with an enlargement of its anterior end, 0.13–0.24 (0.17) mm. in length; prepharyngeal gland, cephalic gland and oesophageal gland present; intestinal caeca smooth and slender, extending almost to posterior end of body; excretory pore terminal; excretory bladder clavate, extending to anterior level of ovary dorsal to ovary, passing dorsal to testes; two small paired diverticula emerge laterally from near its anterior end, extending along inner margins of caeca ventrally to their outer margins to region of oesophagus; genital pore anterior to intestinal bifurcation, halfway between oesophagus and left margin of body; cirrus sac elongated, S-shaped, with its anterior part always protruded, extending to about midway between acetabulum and ovary passing left or dorsal to acetabulum and uterus; its protruded part covered with minute spinules; seminal vesicle spirally sinuous, divided into one main part and three smaller parts within posterior part of cirrus sac; ejaculatory seminal vesicle, ejaculatory duct, prostate cells and cirrus present; testes sub-globular or slightly lobed, post-medial, located close together: anterior testis 0.23–0.53 (0.33) mm. in diameter; posterior testis 0.22–0.48 (0.31) mm. in diameter; vasa deferentia emerging antero-dorsally from testes, entering posterior end of seminal vesicle separately passing ventral to seminal receptacle and Laurer's canal, and dorsal to uterus; ovary anterior to testes, median or sub-medial, with four lobes, 0.07–0.21

(0.13) mm. in length by 0.11–0.29 (0.19) mm. in width; primary oviduct originating from anterior end of ovary, secondary oviduct emerging from seminal receptacle; seminal receptacle large, pear-shaped, dorsal to vitelline reservoir, 0.07–0.15 (0.13) mm. in length by 0.15–0.29 (0.22) mm. in width; Laurer's canal long, sinuous or coiled, emerging from pointed, left end of seminal receptacle, extending laterally to inner left caecal margin, opening dorsally; vitelline reservoir anterior to ovary; coils of uterus intercaecal, between acetabulum and ovary; metraterm present, short, passing to left of cirrus sac; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.06–0.08 by 0.03–0.05 mm., unshrunk eggs in the proximal end of uterus ovoidal, 0.063–0.082 by 0.03–0.05 mm.; vitellaria lateral, well developed, along outer margins of caeca and extending ventrally beyond their inner margins, extending from level of acetabulum to near posterior end of body, forming about four rows posterior to posterior testis; each mass of vitellaria consisting of several vitelline cells; each vitelline cell consisting of one vitelline nucleus and several vitelline follicles.

Sixty specimens were collected from the intestine and stomach of a sculpin, *Endophrys bison* Girard.

Variations: Testes occasionally separated by 0.11 mm. at a maximum; with few vitelline follicles extending to acetabulum, and short interruptions in the continuity of vitellaria at anterior levels of testes frequently present.

Relationships: *P. endophrysi* is similar to *P. atomon* (Rudolphi, 1802) in the shape of body. However, it may be distinguished by the following characters: (1) seminal vesicle sinuous instead of being coiled; (2) anterior part of cirrus sac protruded instead of being not protruded; (3) testes larger, close together instead of being smaller and separated by a considerable distance or by vitellaria.

Discussion: Odhner (1905), pointing out several variations in the shape of body, the length of body, the shape of oral sucker and pharynx, states that vasa deferentia unite with each other before entering the cirrus sac in *P. atomon*. However, they entered the seminal vesicle separately in *P. endophrysi* as well as in several other species in the genus.

The eggs in the proximal end of uterus are not yellowish-brown in color, due to incompletely hardened shell. The eggs with a single longitudinal rib are used for measurements, and those in the proximal end of uterus are used for unshrunk eggs. It is presumed that the process of hardening the shell affects slightly the size of eggs as well as does also the single longitudinal rib.

Podocotyle apodichthysi sp. nov.

Plate I, figs. 1–4, 7 and 10

Description: Body elongated, sub-cylindrical, tapering anteriorly and pointed posteriorly, 1.30–5.34 mm. in length by 0.41–0.80 mm. in width; cuticula smooth; oral sucker terminal, globular in outline, 0.17–0.28 mm. in length by 0.16–0.29 mm. in width; ventral sucker elliptical in outline, slightly embedded or pedunculated, at 0.21–0.35 total length from anterior end, slightly larger than oral sucker, 0.18–0.29 mm. in length by 0.19–0.37 mm. in width; prepharynx 0.03 mm. in length; pharynx

0.11–0.19 mm. in length by 0.12–0.19 mm. in width; oesophagus 0.10–0.23 mm. in length; prepharyngeal gland, cervical gland and oesophageal gland present; intestinal caeca very slender, extending nearly to posterior end of body; excretory pore terminal; excretory bladder slender, elongated, extending to level of ovary dorsally; genital pore at middle level of oesophagus, halfway between oesophagus and left margin of body; cirrus sac elongated, clavate, extending a little posterior to anterior level of acetabulum; its anterior part protrusible; cirrus, ejaculatory duct and prostate cells present; seminal vesicle, within posterior part of cirrus sac, divided into one large main posterior part and one slender coiled anterior part; testes post-medial, only slightly larger than ovary, with 3–6 irregular lobes, separated from each other by 0.06–0.34 mm.: anterior testis 0.15–0.30 mm. in diameter; posterior testis 0.18–0.29 mm. in diameter; ovary closely anterior to testes, with three more or less posteriorly directed lobes, median or sub-medial, 0.09–0.25 mm. in length by 0.10–0.32 mm. in width; seminal receptacle dorsal to left of ovary, pear-shaped, with its pointed part directed anteriorly, but recurving in a hook-shaped end, 0.28 mm. in length by 0.11 mm. in width; Laurer's canal long, coiled, emerging from pointed, left end of seminal receptacle, extending laterally to left caecal margin dorsally as far as the cuticula, opening to outside not determined; shell gland anterior to ovary, diffuse laterally, largely intercaecal; vitelline reservoir ventral to seminal receptacle; coils of uterus intercaecal, extending between acetabulum and ovary; metraterm about half of cirrus sac in length, passing left of cirrus sac; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.06–0.07 by 0.03–0.04 mm., unshrunk eggs ovoidal, 0.07 by 0.05 mm.; vitellaria lateral, well developed, extended along outer margins of caeca and ventrally over inner caecal margins, extending from posterior level of acetabulum nearly to posterior end of body, with interruptions at levels of ovary and testes, arranged in about four rows posterior to anterior testis.

Ten specimens including two young ones were collected from lower part of the intestine of two blennies, *Apodichthys flavidus* Girard.

Variations: Length of body varied in a wide range in sexually matured specimens and size of intestinal caeca also varied in sexually matured specimens, younger specimens possessing larger caeca: Relative position of acetabulum to total length of body varied according to age of animals studied, 0.35 in the specimen of 1.30 mm. in length while 0.21 in the specimen of 5.34 mm. in length. Variation in the shape of ovary in depth of lobes; the distance between testes varied according to age of animals studied, 0.06 mm. in 1.30 mm. specimen while 0.33 mm. in 5.34 mm. specimen. Slight variations were present in the distribution of the almost continuous vitellaria.

Relationships: *P. apodichthysi* is similar to *P. reflex* (Creplin, 1825) in the shape of body. However, it may be distinguished by the following characters: (1) ventral sucker is slightly larger than oral sucker instead of being twice as large; (2) cirrus sac extended not quite to middle level of acetabulum instead of being extended midway between acetabulum and ovary; (3) testes are lobed instead of being smooth; (4) testes are the same size as the ovary instead of being much larger; (5) eggs are smaller, 0.07 mm. in length, instead of being larger, 0.077–0.091 mm. in length.

Discussion: Ova were found in the seminal receptacle and show differences in their structure from those in the distal end of ovary, those in the seminal receptacle, and those in the proximal end of uterus (Figs. 2-4). The behavior of chromosomes has not been observed due to condition of specimen. However, chromosomes seem to diffuse in nucleoplasm in the ova in the proximal end of uterus. This condition may be explained as the result of fertilization. These ova are in a stage prior to cleavage, and their condition supports Odhner's (1905) statement that the development of the eggs starts after discharge in *P. atomon*.

Podocotyle blennicottusi sp. nov.

Plates II and III, figs. 11, 16, 17, 22

Description: Body elliptical in outline, slightly attenuated anteriorly and rounded or conical posteriorly, dorso-ventrally flattened, 1.77-2.02 mm. in length by 0.71-0.74 mm. in width at level of anterior testis; cuticula thick, ridged transversely; rudimentary spines present in region of acetabulum; oral sucker terminal or subterminal, 0.17-0.21 mm. in length by 0.16-0.19 mm. in width; ventral sucker elliptical in outline, at 0.23-0.25 total length from anterior end, about 1.7 times larger than oral sucker, 0.19-0.29 mm. in length by 0.37-0.39 mm. in width; prepharynx visible in sections only; pharynx 0.11-0.17 mm. in length by 0.10-0.11 mm. in width; oesophagus 0.13 mm. in length, indistinct in one specimen; prepharyngeal gland and oesophageal gland present; intestinal caeca slender, extending nearly to posterior end of body; excretory pore terminal and somewhat ventral; excretory bladder broadly clavate, extending nearly to anterior level of ovary, passing dorsal to testes and ventral to seminal receptacle; two small diverticula emerging laterally from nearly its anterior end extend anteriorly in sinuous curves along inner margins of caeca ventral to their outer margins; genital pore at level of pharynx, halfway between pharynx and left margin of body; genital atrium short, lined with cuticula; cirrus sac very elongated, extending nearly to middle of body between acetabulum and ovary; cirrus, ejaculatory duct, ejaculatory seminal vesicle and large and small prostate cells present; seminal vesicle spirally sinuous within posterior part of cirrus sac, constricted into one large main part and a slender part; testes abovoidal, closely tandem, post-median, with irregular margins, intercaecal: anterior testis 0.34-0.38 mm. in diameter; posterior testis 0.35-0.36 mm. in diameter; vasa deferentia emerging dorsally from testes, entering posterior end of seminal vesicle separately close together, the one from anterior testis passing ventral to Laurer's canal and that from posterior testis passing on right side of ovary; ovary closely anterior to anterior testis, sub-median, with four unequal lobes, 0.11-0.13 mm. in length by 0.23-0.27 mm. in width; primary and secondary oviducts present; seminal receptacle large, pear-shaped, dorsal and to left of ovary, its pointed end to left body, 0.10 mm. in length by 0.27 mm. in width; Laurer's canal long, coiled, emerging from pointed, left end of seminal receptacle, extending laterally to region of inner left caecal margin, opening dorsally; gland anterior to ovary, diffuse laterally, largely intercaecal; vitelline reservoir median or sub-median, dorsally anterior to ovary; coils of uterus intercaecal, between acetabulum and anterior testis; metraterm about half length of cirrus sac, to its left; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.065-0.080 (0.073) by 0.034-0.042 (0.036) mm.; vitellaria lateral, well developed, along outer margins of caeca dorsally and ventrally over inner caecal margins, extending from anterior level of acetabulum nearly to posterior end of body forming about four rows posterior to posterior testis; each mass of vitellaria consisting of several vitelline cells; each vitelline cell consisting of one vitelline nucleus and several vitelline follicles.

Four specimens were collected from the lower intestine of a sculpin, *Blennicottus globiceps* Girard.

Variations: The presence of ridges in cuticula varied according to contraction of body; variation in the distribution of vitellaria present, showing signs of interruptions at level of anterior testis.

Relationships: *P. blennicottusi* is similar to *P. levenseni* Isaitschikov (1928) and *P. pearsei* Manter (1934) in the general shape of body. However, it may be distinguished by the following characters: (1) seminal vesicle sinuous instead of being coiled; (2) cirrus sac well extended posterior to acetabulum instead of being extended to its middle level; (3) cuticula transversely ridged instead of being smooth; (4) ovary with four lobes instead of three.

Nomenclature: The terms primary and secondary oviduct are used respectively for the duct from ovary to seminal receptacle and for that from seminal receptacle to ootype, and designate the proximal and distal parts of the oviduct. The term germinal duct used by Yamaguti (1934, 1935) should be abandoned. Ejaculatory seminal vesicle is preferable to ejaculatory sac or ejaculatory pouch as used for *Alaria nasuae* by La Rue and Townsend (1932) for these reasons: (1) it is composed of longitudinal muscle fibers and may have functions both of storing and ejaculating spermatozoa at the time of copulation; (2) it should be distinguished from the seminal vesicle because of its muscular structure although the former may be modified from the latter functionally; (3) some digenetic trematodes as *Hemiurus merus* Linton (1910) and *Parahemiurus australis* Woolcock (1935), possess one seminal vesicle with muscular wall which should be called ejaculatory seminal vesicle because of its supposed function. Vitelline cell and vitelline nucleus are preferred to yolk cell and yolk nucleus as used for *P. ayu* Takahashi (1928) because of its related meaning to vitellaria.

Discussion: The statement by Alvey (1936) that insemination takes place in the distal end of the ovary in *Sphyranna oligorchis* (monogenetic trematode) indicates that there is a doubt in regard to the statement by Faust (1929) that the ootype is a chamber where fertilization takes place in the digenetic trematodes. In *P. apodichthysi* ova were found in the distal end of the ovary, in the seminal receptacle and in the ootype. These ova differ from each other in morphological structures. However, no evidence is available to determine as to whether or not those which have left the primary oviduct have been fertilized. Spermatozoa were found by me in the primary oviduct as well as in Laurer's canal in *P. blennicottusi* and several other species of the genus. Besides, a vitelline cell with one nucleus was found in the seminal receptacle in one of two specimens sectioned. Also several vitelline nuclei with vitelline follicles were found in the seminal receptacle of *P. kofoidi*, although it was not

possible to distinguish them from degenerating ova in this particular case. This fact brings up another question as to whether or not the ootype is the chamber where zygotes always receive vitelline cells. However, no attempt is made to explain this particular instance at the present time. The so-called embryonic cells by Alvey (1936) are found in *P. blennicottusi* as well as several other species of this genus, and these cells are traced to vitelline nuclei easily by means of using Mallory's triple stain. Therefore, these nuclei are called vitelline nuclei instead of being called embryonic cells in *P. blennicottusi*.

The size of eggs is the only reliable difference between *P. levenseni* Isaitschikov (1928) and *P. pearsei* Manter (1934). However, Cort (1915) pointed out the slight variation in the size of eggs in *Pneumonoeces similiplexus* Stafford. Therefore, the validity of *P. pearsei* as a different species from *P. levenseni* can scarcely be established only by a difference in the size of their respective eggs.

Podocotyle californica sp. nov.

Plate II, figs. 12-13

Description: Body elliptical in outline, tapering anteriorly and rounded posteriorly, dorso-ventrally flattened, 2.18-3.85 mm. in length by 1.01-1.40 mm. in width at level of ovary; cuticula thick, ridged transversely; oral sucker subterminal, 0.24-0.30 mm. in length by 0.18-0.25 mm. in width; ventral sucker slightly embedded or pedunculated, at 0.25-0.28 total length from anterior end, 0.30-0.41 mm. in length by 0.45-0.50 mm. in width; prepharynx very short; pharynx 0.16-0.21 mm. in length by 0.11-0.16 mm. in width; oesophagus muscular, 0.06-0.22 mm. in length; prepharyngeal gland and oesophageal gland present; intestinal caeca slender, wavy or smooth, extending nearly to posterior end of body; excretory pore terminal or subterminal; excretory bladder, with an occasional expansion posteriorly, extending to level of ovary; genital pore at level of prepharynx, halfway between prepharynx and left margin of body; cirrus sac clavate, extending only to middle level of acetabulum; seminal vesicle slightly constricted into a posterior large main part and a small anterior part; testes post-median, close together, irregularly lobed: anterior testis 0.45-0.61 mm. in diameter; posterior testis 0.50-0.55 mm. in diameter; ovary closely anterior to anterior testis, submedian, with four unequal lobes, 0.11-0.22 mm. in length by 0.32-0.36 mm. in width; seminal receptacle large, pear-shaped, at left of ovary, 0.12-0.19 mm. in length by 0.28-0.29 mm. in width; Laurer's canal long, coiled, emerging from pointed, left end of seminal receptacle, extending laterally to region of inner left caecal margin; shell gland anterior to ovary, diffuse laterally, largely intercaecal; vitelline reservoir anterior to ovary; coils of uterus intercaecal, between acetabulum and ovary; metraterm long, little shorter than cirrus sac, passing its left; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.063-0.069 by 0.034-0.036 mm.; vitellaria lateral, well developed, extending from middle level of acetabulum to posterior end of body, forming about four rows posterior to posterior testis.

Variations: Cirrus sac extended to anterior, middle, or posterior edge of the acetabulum according to the embedded or pedunculated condition of the acetabulum; vitellaria extended not as far as posterior level of acetabulum, with variable interruptions at level of testes in some speci-

mens; ovary lobed irregularly in one specimen; position of genital pore varied according to contraction or extension of anterior part of body; excretory pore dorsal in two specimens.

Five specimens were collected from the lower intestine of two sculpins, *Blennicottus globiceps* Girard.

Relationships: *P. californica* is similar to *P. blennicottusi* in the shape of body and has the same host. However, it may be distinguished by the following characters: (1) cirrus sac not extending posterior to acetabulum instead of being extended to middle of body; (2) seminal vesicle constricted instead of being spirally sinuous; (3) length of body greater, 2.18–3.85 mm., instead of being 1.77–2.02 mm.; (4) eggs are slightly smaller, 0.063–0.069 mm. in length, instead of being little larger, 0.065–0.080 mm. in length.

It may also be distinguished from *P. levensemi* Isaitschikov (1928) by the following characters: (1) seminal vesicle constricted instead of being coiled; (2) cuticula transversely ridged instead of being smooth; (3) ovary four-lobed instead of being three-lobed; (4) eggs are smaller, 0.063–0.069 mm. in length, instead of being larger, 0.0807–0.0954 mm. in length; (5) testes deeply lobed instead of being smooth.

Discussion: Thick and transversely ridged cuticula is not considered as a character sufficient to exclude *P. blennicottusi* and *P. californica* from this genus.

Podocotyle kofoidi sp. nov.

Plate III, figs. 19, 20 and 23

Description: Body elongated, cylindrical, tapering anteriorly and pointed posteriorly, 3.85–5.48 (4.76) mm. in length by 0.69–1.01 (0.85) mm. in width at level of ovary; cuticula smooth; rudimentary spines present in region of acetabulum; oral sucker terminal, 0.21–0.27 (0.24) mm. in length by 0.21–0.25 (0.23) mm. in width; ventral sucker spherical, embedded or pedunculated, at 0.18–0.23 (0.21) total length from anterior end, about 1.8 times larger than oral sucker, 0.33–0.42 (0.38) mm. in length by 0.34–0.47 (0.41) mm. in width; prepharynx 0.04 mm. in maximum length; cervical glands present lateral to oesophagus; pharynx 0.11–0.17 (0.16) mm. in diameter; oesophagus slender, 0.26–0.40 (0.32) mm. in length; prepharyngeal gland and oesophageal gland present; intestinal caeca slender, extending nearly to posterior end of body; excretory pore terminal; excretory bladder elongated, extending nearly to anterior level of ovary; genital pore anterior to intestinal bifurcation, toward left of body; cirrus sac clavate, extending to posterior level of acetabulum, with an expansion near its protrusible part; protrusible part of cirrus sac covered with minute spinules; seminal vesicle straight, occupying greater part of cirrus sac; prostate cells numerous in the expanded part of cirrus sac; cirrus present; testes subglobular, lobed, post-median, close together in young specimen, in older animals separated by 0.50 mm. at the most: anterior testis 0.33–0.48 (0.40) mm. in diameter; posterior testis 0.38–0.61 (0.43) mm. in diameter; vasa deferentia emerging dorsally from testes, entering posterior end of seminal vesicle separately close together, the one from anterior testis passing toward left of body between ovary and seminal receptacle and that from posterior testis passing toward right of body between ovary and excretory bladder; ovary anterior to testes, median

or submedian, tri-lobed, rarely scarcely lobed or four lobed, 0.17–0.23 (0.20) mm. in length by 0.22–0.29 (0.26) mm. in width; seminal receptacle large, pear-shaped, located dorsally anterior to ovary to left of body, with its anterior pointed end recurved toward left of body; Laurer's canal long, coiled, emerging from pointed, left end of seminal receptacle, running posteriorly along its left side, opening dorsally in region of left caecum at level of ovary; shell gland anterior to ovary, diffuse laterally, largely intercaecal; vitelline reservoir ventral to seminal receptacle; coils of uterus intercaecal between acetabulum and ovary; metraterm little shorter than cirrus sac, passing its left; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.06–0.07 by 0.03–0.04 mm.; vitellaria lateral, well developed, with frequent interruptions at level of testes, along outer margins of caeca dorsally and ventrally beyond the inner caecal margins, extending from near posterior level of acetabulum nearly to posterior end of body, forming about four rows posterior to anterior testes.

Thirty specimens were collected from the intestine of three sculpins, *Blennicottus globiceps* Girard.

Study notes: A mass of spermatozoa was found in the primary oviduct as well as in the Laurer's canal. Many vitelline nuclei and vitelline follicles were found in the seminal receptacle. The cells lining the caeca internally are not conspicuous. The primary oviduct is more muscular than the secondary oviduct. The proximal end of uterus, the so-called ootype, is separated from the secondary oviduct through a slight constriction, and the ootype and the constricted part are covered internally with unicellular glands and externally with shell gland. Connections between most of vitellaria are clearly shown.

Variations: Cirrus sac extended to posterior level of acetabulum when the acetabulum was pedunculated; excretory pore located ventrally or dorsally owing to condition of specimen; opening of Laurer's canal slightly varied in position owing to condition of specimen.

Relationships: *P. kofoidi* is similar to *P. reflexa* (Creplin, 1825) in the cylindrical shape of body. However, it may be distinguished by the following characters: (1) cirrus sac is not extended to posterior level of acetabulum instead of being extended beyond its posterior level; (2) testes lobed instead of being smooth; (3) seminal vesicle straight instead of being coiled.

Podocotyle elongata sp. nov.

Plate III, figs. 18, 21 and 25

Description: Body elongated, slightly dorso-ventrally flattened, tapering anteriorly and bluntly rounded posteriorly, 7.04–7.20 mm. in length by 0.92–0.97 mm. in width at level of acetabulum; cuticula smooth; oral sucker almost terminal, globular in outline, 0.26–0.27 mm. in diameter; ventral sucker sub-globular in outline, at 0.14–0.16 total length from anterior end, about 1.7 times larger than oral sucker in diameter, 0.40–0.42 mm. in length by 0.46–0.47 mm. in width; prepharynx very short; pharynx 0.17–0.18 mm. in length by 0.16–0.17 mm. in width; oesophagus short, 0.14–0.21 mm. in length; prepharyngeal gland, cervical gland and oesophageal gland present; intestinal caeca extended nearly to posterior end of body; excretory pore terminal; excretory bladder elongated, extending to level of ovary; genital pore to left of intestinal bifurcation; cirrus sac clavate, extending nearly to pos-

terior level of acetabulum; its anterior part protrusible; seminal vesicle constricted into a posterior larger part and a smaller anterior part; cirrus protrusible?; testes lobed, post-medial, separated from each other by 0.56–0.65 mm.; anterior testis 0.40–0.41 mm. in diameter; posterior testis 0.43–0.44 mm. in diameter; ovary anterior to testes, with posteriorly directed three lobes, median or submedian, 0.25–0.32 mm. in length by 0.30–0.34 mm. in width; seminal receptacle large, pear-shaped, anterior to left of ovary, with its anterior part bent posteriorly, 0.39–0.41 mm. in length by 0.17–0.21 mm. in width; Laurer's canal long, coiled, emerging from pointed, left of seminal receptacle, extending laterally to region of left caecum, opening dorsally; shell gland anterior to ovary, diffuse laterally, largely intercaecal; vitelline reservoir small, anterior to ovary; coils of uterus intercaecal, extending between acetabulum and ovary; metraterm short, about 0.25 total length of cirrus sac, passing its left; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.057–0.071 by 0.038–0.046 mm.; vitellaria lateral, extending from posterior level of acetabulum nearly to posterior end of body, arranged in about four rows along caeca, with interruptions at level of ovary and testes.

Two specimens were collected from the lower intestine of a sculpin, *Blennicottus globiceps* Girard.

Variations: *P. elongata* is similar to *P. kofoidi* in the shape of body and topography of organs as well as its host. Moreover, the possibility of *P. elongata* being the older stage of *P. kofoidi* is suggested by the following characters: (1) length of body longer; (2) relative position of ventral sucker to body is shorter, 0.14–0.16, instead of being longer, 0.18–0.23; (3) separation between testes greater, 0.65 mm., instead of being shorter, 0.50 mm. However, it may be distinguished by the following characters: (1) oesophagus shorter, 0.14–0.21 mm. instead of being longer, 0.26–0.40 mm. in length; (2) seminal vesicle constricted instead of being straight; (3) ventral sucker less pedunculated instead of being more pedunculated.

Discussion: The length of oesophagus as a specific character in this genus may be questioned. However, since Manter (1926) considers the length of oesophagus as a diagnostic character separating *P. atomon* from *P. olssoni*, I have used it to distinguish *P. elongata* from *P. kofoidi*.

Podocotyle pedunculata sp. nov.

Plate III, fig. 24

Description: Body elongated, cylindrical, bent dorsally in accord with pedunculation of the acetabulum, 1.66–2.30 mm. in length by 0.18–0.41 mm. in width at level of ovary; cuticula smooth; oral sucker terminal or subterminal, 0.11–0.13 mm. in diameter; ventral sucker strongly pedunculated, projecting nearly equal to the dorso-ventral diameter of the body, about at 0.26 total length from anterior end, 0.17–0.22 mm. in diameter; prepharynx present; pharynx 0.07–0.09 mm. in diameter; oesophagus slender, 0.08–0.11 mm. in length; cervical gland present; intestinal caeca slender, extending to posterior end of body; excretory pore terminal; excretory bladder elongated, extending to anterior level of ovary; genital pore at posterior level of pharynx; cirrus sac clavate, extending posteriorly to level of acetabulum, with an expansion near its protrusible part; its anterior part protrusible; seminal vesicle straight; testes circular in outline or at most very slightly lobed, post-medial, separated by about 0.20 mm. from each other: anterior

testis 0.11–0.15 mm. in diameter; posterior testis 0.11–0.13 mm. in diameter; ovary anterior to testes, median or submedian, with three posteriorly directed lobes, 0.07–0.09 mm. in length by 0.08–0.11 mm. in width; seminal receptacle large, pear-shaped, with its pointed part directed anteriorly, but recurving in a hook-shaped end, 0.28 mm. in length by 0.11 mm. in width; Laurer's canal long, coiled, emerging from pointed, left end of seminal receptacle, extending laterally, opening dorsally in region of left caecum; shell gland anterior to ovary, diffuse laterally; coils of uterus intercaecal, between acetabulum and ovary; metraterm long, a little shorter than cirrus sac, passing its left; eggs yellowish-brown, few, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.06–0.063 by 0.030–0.034 mm; vitellaria lateral, tending to be seriated in a single row anteriorly, with interruptions at level of testes, forming four rows posterior to anterior testis.

Six specimens including an immatured one were collected from the intestine of two sculpins, *Oligocottus maculosus* Girard. Preserved specimens were altered from their usual form due to the projecting acetabulum.

Variation: The position of the genital pore to the right of the oesophagus was due to the flattening of the specimen.

Relationships: *P. pedunculata* is similar to *P. kofoidi* in the shape of body and topography of organs. However, it may be distinguished by the following characters: (1) the size of body is smaller, 1.66–2.30 mm. in length instead of 3.85–5.48 mm. in length; (2) testes are smooth or slightly lobed instead of being deeply lobed; (3) ventral sucker is more pedunculated instead of being less pedunculated.

Discussion: *P. pedunculata* might be regarded as the young of *P. kofoidi*. However, it is distinguished by the following characters: (1) body smaller 2.30 mm. in length, than the immatured specimen of the latter, 3.89 mm. in length; (2) testes are separated by more than their diameter instead of being close together as in a young specimen of *P. kofoidi*. If there is any possibility that *P. pedunculata* and *P. kofoidi* are same species, then the differences are due to different hosts. This argument can not be settled without understanding their respective life histories. At the present time there is no basis for placing these two in the same species.

Podocotyle pacifica sp. nov.

Plate 11, figs. 14–15.

Description: Body elongated, tapering anteriorly and bluntly rounded posteriorly, 3.55 mm. in length by 0.73 mm. in width at level of acetabulum; cuticula thick, rather smooth; oral sucker terminal, 0.25 mm. in length by 0.21 mm. in width; ventral sucker elliptical in outline, at 0.22 total length from anterior end, 0.34 mm. in length by 0.40 mm. in width; prepharynx very short; pharynx 0.12 mm. in length by 0.11 mm. in width; oesophagus 0.12 mm. in length; prepharyngeal gland, cervical gland and oesophageal gland present; intestinal caeca extended nearly to posterior end of body; excretory pore terminal; genital pore at level of pharynx, halfway between pharynx and left margin of body; cirrus sac elongated, extended posterior to acetabulum; seminal vesicle sinuous, constricted into one large posterior part and

a small anterior part; cirrus, ejaculatory duct, ejaculatory seminal vesicle and prostate cells present; testes abovoidal, separated by vitellaria: anterior testis 0.38 mm. in diameter; posterior testis 0.34 mm. in diameter; ovary closely anterior to anterior testis, with four sub-equal lobes, submedian, 0.22 mm. in length by 0.27 mm. in width; seminal receptacle large, pear-shaped, antero-dorsal to ovary; Laurer's canal long, coiled, emerging from pointed, left end of seminal receptacle, extending laterally to region of left caecum; shell gland anterior to ovary, diffuse laterally, largely intercaecal; vitelline reservoir large, anterior to ovary; metraterm about one-third of cirrus sac in length, passing its left; eggs yellowish-brown, longitudinally ribbed (due to shrinkage?), ellipsoidal, 0.071–0.078 by 0.034–0.046 mm.; vitellaria well developed, largely lateral, extending from region of acetabulum nearly to posterior end of body, almost meeting posterior to anterior testis, with slight sign of interruptions at level of testes.

One specimen was collected from the intestine of a sculpin, *Blennicottus globiceps* Girard.

Relationships: *P. pacifica* is similar to *P. olssoni* Odhner (1905) in the shape of body and topography of organs. However, it may be distinguished by the fact that the seminal vesicle is sinuous instead of being coiled.

General discussion: The female reproductive system, studied by me in 8 new species of *Podocotyle* and *P. atomon* (?) limited to a single mutilated specimen from *Enophrys bison*, has certain basic similarities in pattern to that of *P. ayu* Takahashi (1928). Therefore, this system may be considered as affording important generic characters. Because of this agreement *P. ayu* should not be excluded from *Podocotyle* because of the extension of its excretory bladder only to the middle level of the posterior testis. For the same reason *Podocotyloides petalophallus* Yamaguti (1934) should be included in *Podocotyle* although its excretory bladder is extended far anterior to the posterior level of the acetabulum. The peculiarly modified genital structures in *P. petalophallus* should not be considered as adequate for a separate genus, but only as specific characters.

KEY TO SPECIES OF *PODOCOTYLE*

- | | | |
|--------|--|----------------------------------|
| 1(10) | Seminal vesicle straight or constricted | 2 |
| 2(5) | Testes very close together | 3 |
| 3(4) | Testes with smooth margins | <i>P. ayu</i> Takahashi (1928) |
| 4(3) | Testes with deeply lobed margins | <i>P. californica</i> sp. nov. |
| 5(2) | Testes separated by a considerable distance or by vitellaria | 6 |
| 6(7) | Testes with smooth margins | <i>P. pedunculata</i> sp. nov. |
| 7(6) | Testes with deeply lobed margins | 8 |
| 8(9) | Oesophagus about twice as long as pharynx | <i>P. kofoidi</i> sp. nov. |
| 9(8) | Oesophagus about same length as pharynx | <i>P. elongata</i> sp. nov. |
| 10(17) | Seminal vesicle sinuous | 11 |
| 11(14) | Testes very close together | 12 |
| 12(13) | Anterior part of cirrus sac protruded | <i>P. enophrysi</i> sp. nov. |
| 13(12) | Anterior part of cirrus sac not protruded | <i>P. blennicottusi</i> sp. nov. |
| 14(11) | Testes separated by a considerable distance or by vitellaria | 15 |
| 15(16) | Excretory bladder extended to the level of the ovary ... | <i>P. pacifica</i> sp. nov. |
| 16(15) | Excretory bladder extended to the posterior level of the acetabulum. | |

P. petalophallus Yamaguti (1934)

- 17(1) Seminal vesicle coiled 18
 18(27) Testes very close together 19
 19(24) Cirrus sac extended to midway of acetabulum 20
 20(23) Testes with smooth margins 21
 21(22) Vitellaria extended to midway of acetabulum.
 P. levenseni Isaitschikov (1928)
 22(21) Vitellaria extended to posterior level of acetabulum.
 P. pearsei? Manter (1934)
 23(20) Testes with deeply lobed margins.
 24(19) Cirrus sac extended to posterior level of acetabulum 25
 25(26) Testes with smooth margins *P. odhneri* Isaitschikov (1928)
 26(25) Testes with deeply lobed margins *P. lanceolata* Price (1934)
 27(18) Testes separated by a considerable distance or by vitellaria 28
 28(31) Cirrus sac not extended posterior to acetabulum 29
 29(30) Testes with smooth margins and much larger than ovary.
 P. syngnathi Nicoll (1913)
 30(29) Testes with lobed margins and about same size as ovary.
 P. apodichthysi sp. nov.
 31(28) Cirrus sac extended posterior to acetabulum 32
 32(35) Oesophagus about twice as long as pharynx 33
 33(34) Vitelline follicles not extended to acetabulum .. *P. atomon* (Rudolphi, 1802)
 34(33) Few vitelline follicles extended anterior to acetabulum.
 P. atomon var. *dispar?* Nicoll (1909)
 35(32) Oesophagus about length of pharynx 36
 36(37) Body dorso-ventrally flattened *P. olssoni* Odher (1905)
 37(36) Body cylindrical *P. reflexa* (Creplin, 1825)

In this key *P. reflexa* (Creplin), *P. levenseni* Isaitschikov, *P. odhneri* Isaitschikov, and *P. lanceolata* Price are considered to possess coiled seminal vesicles though this is not shown clearly in their descriptions. *P. pennelli* Leiper and Atkinson (1914) is not placed in the key due to incomplete description.

SUMMARY

1. Eight new species of *Podocotyle* Odhner are described from tide pool fishes from Dillon's Beach, California.

2. In nomenclature the terms primary oviduct and secondary oviduct are used to determine the proximal and distal parts of the oviduct; vitelline cell is used instead of yolk cell; ejaculatory seminal vesicle is used instead of ejaculatory pouch or merely seminal vesicle with muscular wall; the term germinal duct (Yamaguti, 1934, 1935) should be abandoned.

3. "Embryonic cells" of Alvey (1936) in *Sphyranna oligorchis* are interpreted as vitelline nuclei.

4. *Podocotyloides* Yamaguti (1934) is included in *Podocotyle*.

5. A revision of the genus and a key to the species of *Podocotyle* have been attempted.

LITERATURE CITED

- Alvey, C. H. 1936.—The morphology and development of the monogenetic trematode *Sphyranna oligorchis* (Alvey, 1933) and the description of *Sphyranna polyorchis* n. sp. Parasit., 28: 229–253, pls. 10–11.
 Cort, W. W. 1915.—Egg variation in a trematode species. Jour. Parasit., 2: 25–26.

- Faust, E. C. 1929.—Human Helminthology. (Philadelphia, Lea and Febiger.) p. 74.
- Isaitschikov, I. M. 1928.—Zur Kenntniss der parasitischen Würmer einiger Gruppen von Wirbeltern der russischen Arktis. Berichte Wissenschaft. Merresinstitut., 3: 5-79.
- LaRue, G. R., and Townsend, E. W. 1932.—A morphological study of *Alaria nasuae* LaRue and Townsend (Trematoda: *Alariidae*). Tran. Amer. Micr. Soc., 51: 253-263.
- Leiper, R. T., and Atkinson, E. L. 1914.—Helminthes of the British Antarctic Expedition, 1910-1913. Proc. Zool. Soc. London, 1914, 1-2: 224-226.
- Linton, E. 1910.—Helminth fauna of the Dry Tortugas, 11: Trematodes. Carnegie Inst. Wash. Pub. no. 133, papers Tortugas Laboratory, 4: 15-98.
- Manter, H. W. 1926.—Some North American fish trematodes. Ill. Biol. Monogr. 10: 1-138.
1934. Some digenetic trematodes from deep-water fishes of Tortugas, Florida. Carnegie Inst. Wash. Pub. no. 435, papers Tortugas Laboratory, 28: 261-345.
- Nicoll, W. 1909.—Studies on the structure and classification of the digenetic trematodes. Quart. Jour. Micr. Sci., 53: 391-486.
- 1913.—New trematode parasites from fishes on the English channel. Parasit., 5: 238-246.
- Odhner, Th. 1905.—Die Trematoden des arktischen Gebietes. Fauna Arct., 4: 291-394.
- Price, E. M. 1934.—New digenetic trematodes from marine fishes. Smith. Misc. Coll., 91: 1-8.
- Takahashi, S. 1928.—On a new trematode *Podocotyle ayu* n. sp. from the intestine of *Plecoglossus altivelis* (T and S). Okayama, Medizinische Universität, 1: 51-56.
- Woolcock, V. 1935.—Digenetic trematodes from some Australian fishes. Parasit., 27: 310-331.
- Yamaguti, S. 1934.—Studies on the helminth fauna of Japan, trematodes from fishes, I. Japan. Jour. Zool. Tokyo, 5: 249-541.

EXPLANATION OF PLATES

Genus *Podocotyle* Odhner

PLATE I

- Fig. 1. Egg in the uterus of *P. apodichthysi* n. sp. $\times 646$.
- Fig. 2. Ovum in the distal end of ovary of *P. apodichthysi*. $\times 646$.
- Fig. 3. Ova in the seminal receptacle of *P. apodichthysi*. $\times 646$.
- Fig. 4. Zygote in the proximal end of uterus of *P. apodichthysi*. $\times 646$.
- Fig. 5. Cross section through ejaculatory seminal vesicle of *P. endophrysi* n. sp. $\times 400$.
- Fig. 6. Diagram of genital structures of *P. endophrysi*. $\times 160$.
- Fig. 7. Diagram of cirrus sac of *P. apodichthysi*. $\times 117$.
- Fig. 8. Whole mount of *P. endophrysi* n. sp., ventral view. $\times 35$.
- Fig. 9. Diagram of cirrus sac of *P. endophrysi*. $\times 100$.
- Fig. 10. Whole mount of *P. apodichthysi* n. sp., ventral view. $\times 29$.

PLATE I

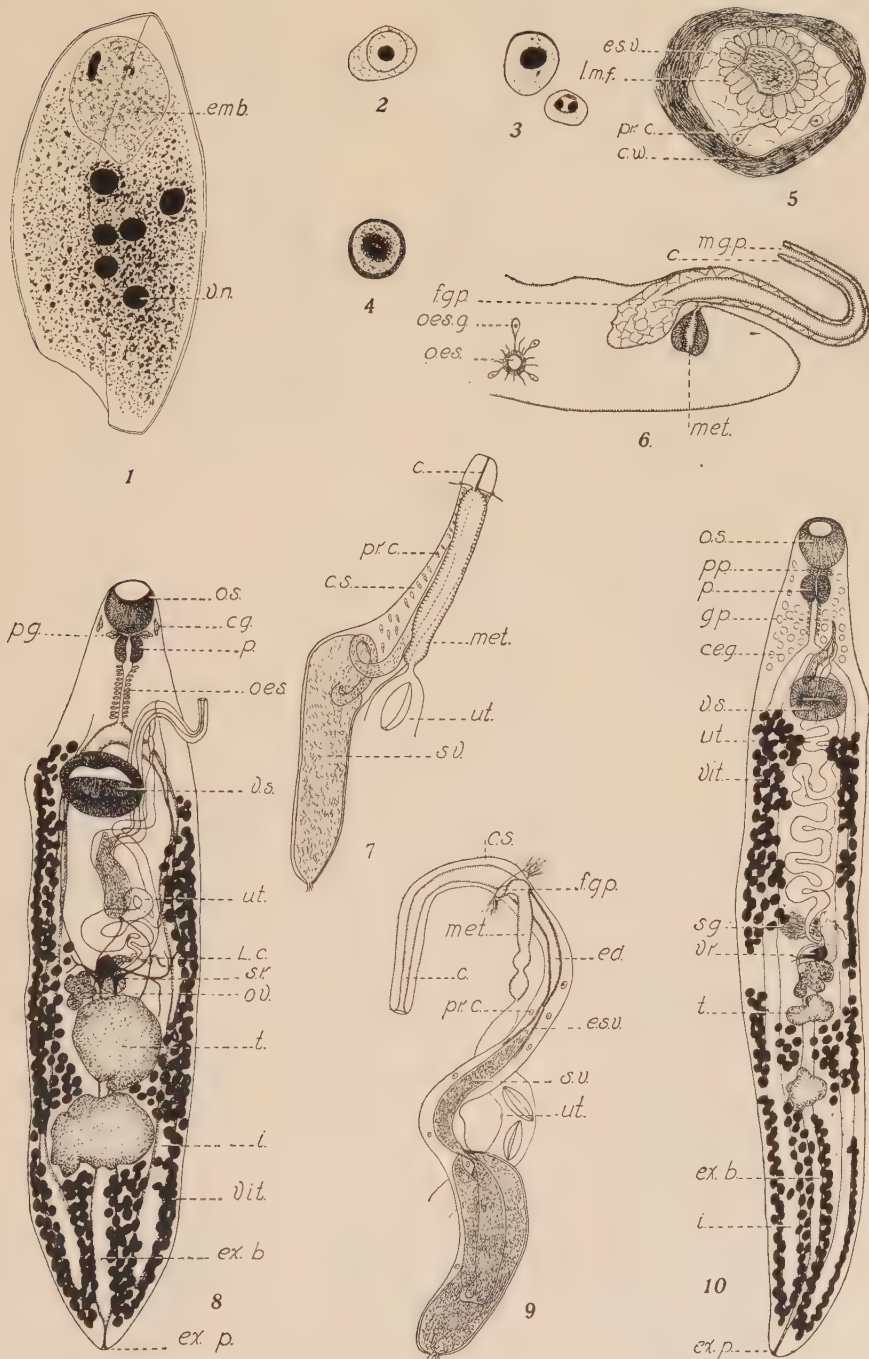


PLATE II

- Fig. 11. Diagram of female reproductive system of *P. blennicottusi* n. sp.
 Fig. 12. Diagram of cirrus sac of *P. californica* n. sp. $\times 116$.
 Fig. 13. Whole mount of *P. californica* n. sp., ventral view. $\times 35\frac{1}{2}$.
 Fig. 14. Diagram of cirrus sac of *P. pacifica*. $\times 73$.
 Fig. 15. Whole mount of *P. pacifica* n. sp., ventral view. $\times 33$.
 Fig. 16. Diagram of cirrus sac of *P. blennicottusi*. $\times 137$.
 Fig. 17. Whole mount of *P. blennicottusi* n. sp., ventral view. $\times 40$.

PLATE III

- Fig. 18. Abnormally constricted caecum of *P. elongata* n. sp. $\times 41\frac{1}{2}$.
 Fig. 19. Diagram of cirrus sac of *P. kofoidi* n. sp. $\times 136$.
 Fig. 20. Longitudinal section of cervical glands of *P. kofoidi*. $\times 768$.
 Fig. 21. Diagram of cirrus sac of *P. elongata*. $\times 81\frac{1}{2}$.
 Fig. 22. Egg in the uterus of *P. blennicottusi*. $\times 740$.
 Fig. 23. Whole mount of *P. kofoidi* n. sp., ventral view. $\times 20\frac{1}{2}$.
 Fig. 24. Whole mount of *P. pedunculata* n. sp., ventral view. $\times 43\frac{1}{2}$.
 Fig. 25. Whole mount of *P. elongata* n. sp. $\times 21$.

Abbreviations: *c.*, cirrus; *c.g.*, cephalic gland; *c.s.*, cirrus sac; *c.w.*, cirrus sac wall; *ce.g.*, cervical gland; *e.d.*, ejaculatory duct; *e.s.v.*, ejaculatory seminal vesicle; *emb.*, embryo; *ex.b.*, excretory bladder; *ex.p.*, excretory pore; *f.g.p.*, female genital pore; *g.a.*, genital atrium; *g.p.*, genital pore; *i.*, intestinal caecum; *k.*, karyosome; *L.c.*, Laurer's canal; *l.m.f.*, longitudinal muscle fibers; *m.g.p.*, male genital pore; *met.*, metraterm; *n.*, nucleus; *nu.*, nucleolus; *o.s.*, oral sucker; *oes.*, oesophagus; *oes.g.*, oesophageal gland; *oot.*, ootype; *ov.*, ovary; *p.*, pharynx; *p.g.*, pharyngeal gland; *p.od.*, primary oviduct; *p.p.*, prepharynx; *pr.c.*, prostate cell; *s.g.*, shell gland; *s.od.*, secondary oviduct; *s.r.*, seminal receptacle; *s.v.*, seminal vesicle; *t.*, testis; *ut.*, uterus; *v.f.*, vitelline follicle; *v.n.*, vitelline nucleus; *v.r.*, vitelline reservoir; *v.s.*, ventral sucker; *vit.*, vitellaria.

PLATE II

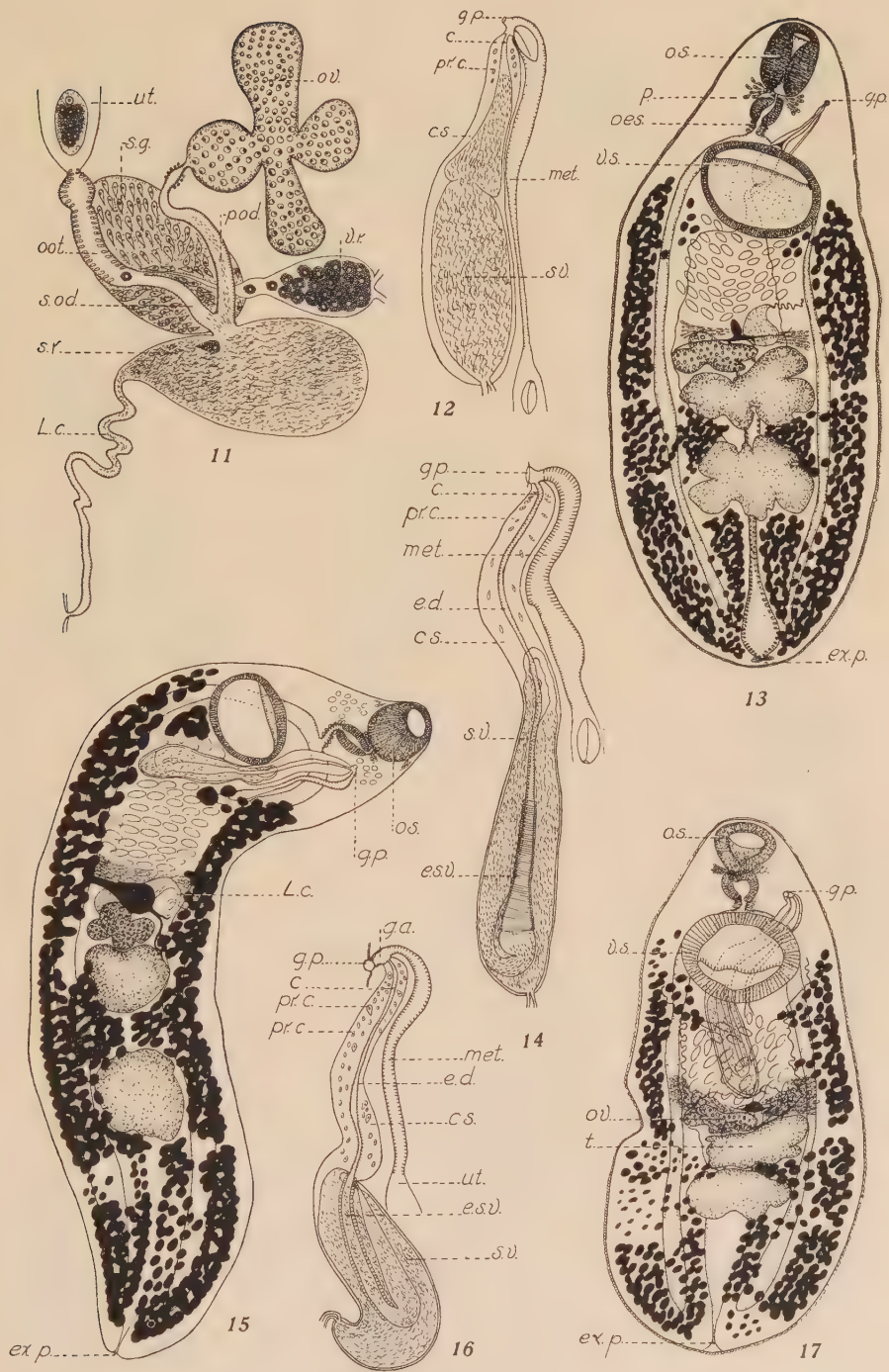
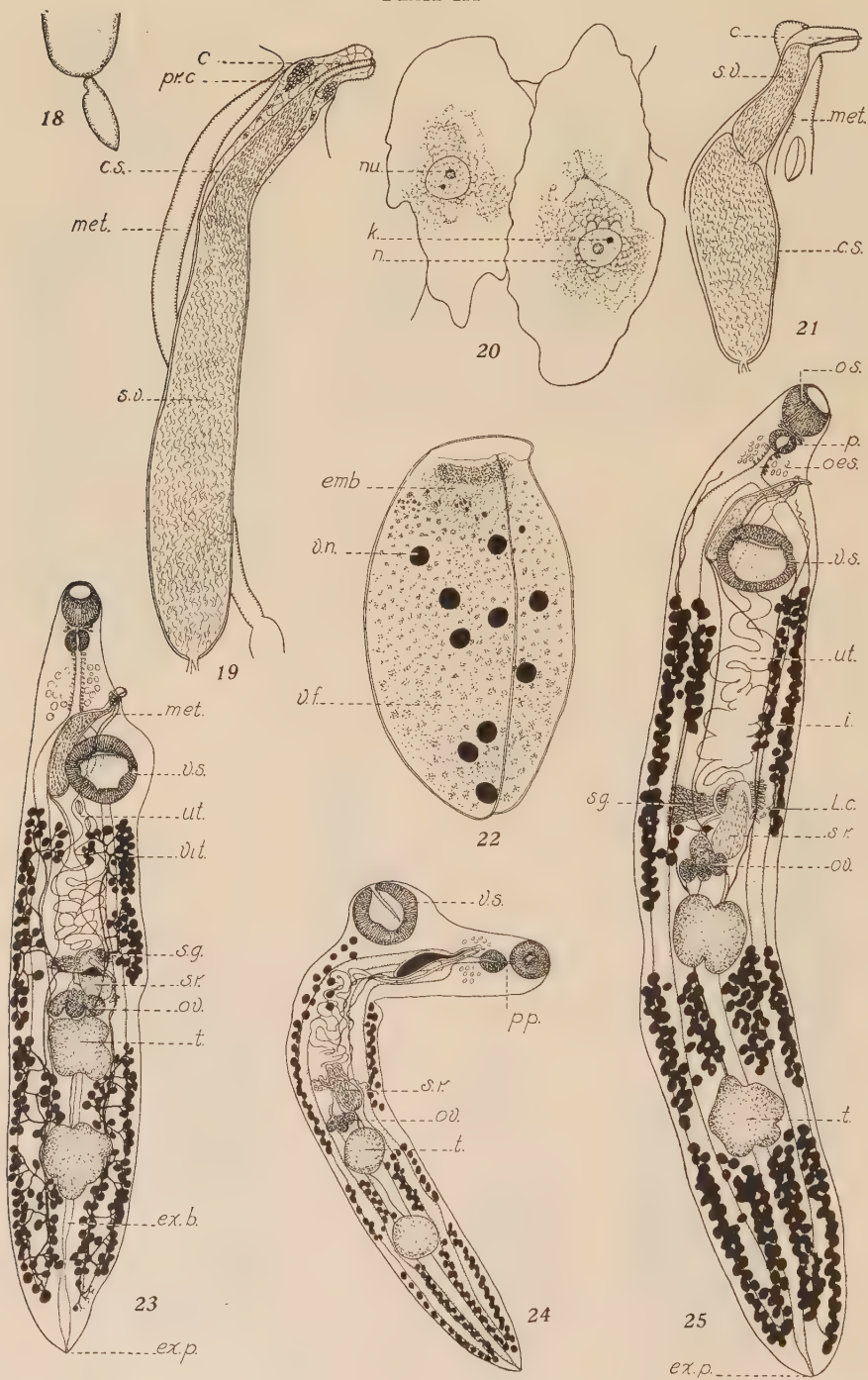


PLATE III



RESEARCH NOTES

EXPERIMENTS ON REGENERATION IN THE TREMATODE, *ECHINOSTOMA REVOLUTUM*

In a recent study of morphological variations in *Echinostoma revolutum* (1937, Ill. Biol. Monogr., 15: No. 1. In press), I observed diminutive, duplicated, and malformed structures, a number of which recalled similar conditions observed in other forms where they were known to have arisen from traumata and regeneration. The present report is a brief statement of the results of a series of preliminary experiments in which I have attempted to reproduce some of those abnormal conditions, and to determine the general nature and degree of repair following six different types of mechanical injury.

Fifty-eight adult worms (10-17 days of age) were taken from experimentally infected pigeons, operated, and re-introduced into the cloaca of recipient pigeons. They were recovered either from the stools or from the gut of sacrificed hosts.

Worms with incisions through the body wall of the pre-acetabular region, or extensive lacerations of the head-crown, were expelled within five hours and showed no appreciable repair. Only 6 operations of this type were performed although a portion of the next group more or less falls into this class.

From 1 to 12 cephalic spines were removed from 15 individuals. Since considerable tissue damage was unavoidable in most cases, only 5 worms remained in the recipient host more than 24 hours. One from which 12 of the dorsal spines were extracted, lived 9 days after operation. Although the tissues appeared to be entirely normal in this specimen, no indication of spine regeneration could be observed.

Median dorso-ventral slightly lacerated septic puncture wounds through the middle of the body were produced on 5 individuals. The wound was completely healed after 2 days.

The highest degree of repair was discovered in worms with deep unilateral incisions through the body wall at the level of the testes. The incision severed the cecum of the digestive system, the vitellaria, and the excretory siphon. Of the 10 worms operated in this manner, 9 were allowed to live 7 days in the recipient host. These worms not only repaired the injury, but also continued to grow and to generate ova. However, the repair was not yet completed. The wound was closed with a furrow-like scar which was covered by a thick layer of tuberosc cuticula. The cut ends of the cecum were closed and the isolated portion was normal in appearance. Anterior to the lesion, the excretory siphon became much enlarged and muscular to form a bladder with an opening to the outside at the site of the wound. Due to degeneration in the posterior field of the vitellaria, the hind body became diminutive on the operated side. Manter (1930. Carnegie Inst. Year Book No. 29: 338-340) observed a similar condition in a specimen of *Helicometra torta*, in which the wound was completely healed, the cut ends of the cecum had closed, and the isolated portion of the follicles posterior to the lesion and the posterior testis were in advanced stages of degeneration, the latter having almost disappeared. The conditions within the excretory system, the cuticula, and the deeper tissues surrounding the point of injury were not described.

Amputation of the hind portion of the body is followed by an immediate contraction of the body wall to partially close the wound. There is then a rapid secretion of a deep layer of cuticula over the cut surface and a very conspicuous aggregation of cells at the site of the injury. In this case as well as all others in which the body wall is broken, the cell aggregates are proliferated for the most part from the submuscular layer of the body wall. Infiltration of cells from the surrounding parenchyma may also contribute to these aggregates. There is a gradual growth or folding inward of the cut margins of the body wall to entirely close the wound in about three days. An outlet for the excretory bladder is established and the cut ends of the intestine become closed. The anterior parts of the

body remain functional and normal in appearance. Twenty-two worms were operated in this manner, the longest post-operative period in the recipient host being 115 hours.

In drawing conclusions regarding the speed with which repair proceeds in this parasite, it is important to bear in mind that its normal development is fairly rapid. In the pigeon it begins oviposition as early as eight days after infection. During that period there is an enormous increase in size, and the entire complex of generative organs is differentiated and matured. It continues its growth after maturity and usually lives only three or four weeks in the pigeon.—PAUL BEAVER, *Oak Park Junior College, Oak Park, Illinois.*

NOTES ON THE BLACK WIDOW SPIDER IN THE SAN JOAQUIN VALLEY

Observations by the writer during the last 5 years suggest that the black widow spider, *Latrodectus mactans* F., has been increasing in the San Joaquin Valley of California, at least in and about farm buildings located in raisin vineyards.

Formerly the general practice was to dry grapes on wooden trays, but during recent years there has been a change in the method of drying Thompson Seedless (Sultanina) raisins. Wooden trays have been replaced to a considerable extent by paper trays, which last only one season. In the 1936 season about 100,000 tons of Thompson Seedless raisins were dried on papers. When wooden trays are used, they are stacked in piles between the rows of vines, after the grapes are partially dried. The paper sheets, however, are rolled or folded in various ways and left on the ground with a layer of raisins on them, for further drying of the fruit. After being folded or rolled, paper trays are a favored shelter for black widow spiders, and the more general use of these trays seems to explain the increase in the numbers of spiders observed.

In the laboratory this spider thrives on a diet of larvae of the raisin moth, *Ephestia figulilella* Gregson. The presence of the larvae in the fruit enclosed in paper trays, and of female moths attempting to enter the trays for egg laying, probably influences the spiders' choice of shelter, as well as the further reason that the paper trays offer better opportunities for concealment in their crumpled or roughly folded ends.

In 1936 the writer found no black widow spiders in 200 wooden trays stacked in 2 locations. During the same season 1,100 paper trays from 10 different crops of the Thompson Seedless variety were opened and examined and 29 black widow spiders were recorded. Since about 36,000,000 papers probably were used in drying the crop, the estimated infestation in the paper trays was about 936,000 black widow spiders. This figure may be less than the total which frequented the trays, since typical webbing was found in many trays in which there were no spiders. No doubt some escaped because of fright during the handling of trays. It is assumed that, late in the season, others abandoned their webs on trays in search for more secluded overwintering quarters.

Raisins from wooden trays are boxed in the vineyards. To a considerable extent the paper trays of fruit are hauled to the farmyards for boxing. The latter procedure, advantageous in some respects, concentrates the spiders in the farmyards where they doubtless enter outbuildings, boxes, wood piles, and similar shelters, thus increasing the numbers in and about farm buildings.—HEBER C. DONOHUE, *Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.*

ERYTHROCYTE COUNTS AND HEMOGLOBIN CONCENTRATION IN NORMAL FEMALE CANARIES

There is very little information in the literature on this subject. Since the female canary is widely used for experimental studies on bird malaria, it seems

desirable to determine the normal values of the oxygen-carrying components of the blood. Ben-Harel (1923, *Amer. Jour. Hyg.* 3: 652-686) in this laboratory found the normal erythrocyte count "to range from 4.5 million to a little over 5 million, the mean being somewhere around 4.5 million." Although not so stated in her paper, these counts must have been made on female canaries since males are not ordinarily used here for experimental purposes.

The present work was done on healthy mature female canaries during the fall, winter, and early spring months. Inasmuch as the nucleated red corpuscles of canaries hemolyze slowly, a technique slightly different from that used in the determination of human hemoglobin was employed. For hemoglobin determinations, the blood was drawn from the leg vein into a standardized hemoglobin pipette to the 20 cu. mm. mark and diluted with tap water. This was mixed well and allowed to stand for two hours to insure complete hemolysis. Then 5.02 cc. of 5/N HCl was added which gave a final dilution of 10/N HCl. Six hours later, or when the cell stroma and nuclei had settled to the bottom, the hemoglobin concentration was read on a Newcomer hemoglobinometer. The hemoglobin concentration was read in percentages and then converted into grams using 17.7 grams of hemoglobin per 100 cc. as 100%. The erythrocyte counts were determined by the usual method using Hayem's diluent, a Thoma pipette standardized by the U. S. Bureau of Standards, and counted in a Levy Counting Chamber with the new Neubauer ruling. At least two counts were made of each sample and if these did not check, counts were repeated until the readings agreed.

Nineteen hemoglobin determinations were made and gave a range from 7.08 grams to 12.04 grams with a mean of 9.50 grams per 100 cubic centimeters of blood. The erythrocyte count was determined in 23 birds which gave a range from 3,500,000 to 5,700,000 per cubic millimeter. The mean red cell count was 4,516,000 per cubic millimeter.

TABLE I
ERYTHROCYTES PER CUBIC MILLIMETER OF BLOOD

Month	Number of Birds	Mean	σ Mean	Diff. σ Diff.
Nov.-Dec.	12	4,320,000	165,862	1.87
March	11	4,729,000	143,345	
Total	23	4,516,000	115,872	

HEMOGLOBIN IN GRAMS PER 100 CC. OF BLOOD

Month	Number of Birds	Mean	σ Mean	Diff. σ Diff.
Nov.-Dec.	8	8.14	.299	5.71
March	11	10.98	.281	
Total	19	9.50	.349	

Riddle and Braucher (1934, *Amer. Jour. Physiol.* 108: 544-566) studied the erythrocyte and hemoglobin variations in pigeons and doves. From November to May these birds were kept in glass cages to exclude the ultra-violet light. They found that the hemoglobin was highest in winter and lowest in summer. The summer decrease was correlated with the exposure to ultra-violet rays and to air of least cooling power. In the winter when the hemoglobin was highest the birds had not been exposed to ultra-violet light nor to air of greatest cooling capacity (autumn). They also found that the erythrocytes were highest in autumn and lowest in summer.

The birds in the experiment reported in this paper were kept under normal laboratory conditions. One group was bled in late November and early December and another group during March. As shown in Table I the March group gave higher values for both hemoglobin and erythrocytes. These differences were tested to find if they were statistically significant. The difference between the erythrocytes (1.87 standard deviations) is not significant. However, the hemo-

globin of the March group is significantly higher (5.17 standard deviations) than the Nov.-Dec. group.—MARTIN D. YOUNG, *Department of Protozoology, School of Hygiene and Public Health, The Johns Hopkins University.*

AN APPARENT CASE OF SEXUAL DIFFERENCE IN RESISTANCE TO PARASITIC INFECTION

There seems to be little mention in scientific literature in respect to sexual differences in resistance to parasitic infections. It seems therefore worth while to record such a situation in regard to the presence of *Syngamus trachea* in Hungarian partridge (*Perdix perdix perdix*) at the Michigan State Game Farm.

During the past year a total of 34 cases of syngamiasis has been brought to autopsy in the partridges coming from the State Game Farm. About half of these were mortalities due to the parasitic infection. The remainder were due to other causes. In the 34 records there has been a remarkable disparity in the representation of sex in the birds. Thirty-two have been female birds. Only two males have been found to harbor the parasites. The chances for either sex to pick up the parasites have apparently been equal as far as can be determined. They were confined in breeding pens in pairs, with no segregation of sexes.

One can only speculate as to the reason for this sexual difference in the degree of infection with *Syngamus trachea* in this group of Hungarian partridges. A reasonable explanation might be that the strain of egg production in the females lowered their resistance sufficiently to allow the parasite to establish itself.—S. C. WHITLOCK, *Game Division, Michigan Department of Conservation.*

EXCYSTATION OF THE COCCIDIA, *EIMERIA TENELLA*

Very little has been written about the freeing of the sporozoites from the oocyst of coccidia of birds. Hadley, Fantham, Becker and others state that the excystation is brought about by the effect of the pancreatic juices or enzymes acting in the lumen of the duodenum. Hadley (1911, Archiv f. Protistenk. 23: 7) says that an occasional infection of the mucous membrane of the mouth, throat, crop and proventriculus may occur. That might be construed to mean that excystation could take place elsewhere than in the lumen of the duodenum.

Becker (1934, Coccidia and Coccidiosis . . ., Collegiate Press Inc., Ames, Iowa) states that the sporozoite is liberated from the spore and depicts it making its way through the wall of the oocyst at the region of the micropyle at one end of the oocyst. Hadley, however, saw spores ejected from the oocyst at the region of the micropyle. Observations made during the course of this study lead one to conclude that the spores are regularly freed from the oocyst before the sporozoites break from the spores. The spores have been seen coming through the side of the oocyst wall and the freed spores were always much more numerous than were the sporozoites. No sporozoite has been seen freed from the spore while still within the unruptured oocyst. The freeing of the sporozoites from the spore has not yet been observed.

Observations were made to determine the place or places in the digestive tract at which excystation takes place. Contrary to the statements in the literature, it has been found that freeing of the spores from the oocysts and the liberating of the sporozoites may occur in the crop. In a total of eight chickens upon three different occasions spores or sporozoites were demonstrated in material recovered from the crop.

In some experiments only five minutes were required to bring about excystation in the crop as demonstrated by the presence of spores and sporozoites.

To demonstrate that passage through the crop and proventriculus is not necessary for excystation to take place, the duodenum of a chicken was ligated just below the gizzard. Sporulated oocysts were then introduced into the lumen

of the duodenum with a hypodermic needle. Twenty minutes later the material was recovered from the lower one-third of the intestine. Live spores were numerous and in this material spores were seen in two cases escaping from oocysts. Oocysts from which a spore has escaped present a "caved-in" appearance on one side of the cyst.

In order to be certain that enzymes from further down the digestive tract had not been forced up into the crop and had brought about the excystation there, the crops of two chickens were resected from the proventriculus, washed out with warm saline and the lower opening ligated. Sporulated oocysts were introduced into the crop and after an hour the material was removed to a dish and examined for the presence of excysted forms. Both spores and sporozoites were present, although in very small numbers as compared with the apparently unchanged oocysts.

Numerous attempts have been made to excyst this parasite *in vitro*, but they have been without significant success. Water extracts of crop, proventriculus, duodenum and pancreas have been tried. Chicken bile and the fluids found in the lumen of the crop and of the duodenum were tested. A water solution of commercial pancreatin was tried. To this was added chicken bile or 0.5% sodium carbonate to attain the alkalinity normally found in the duodenum of the chicken. No excystation was observed.—IVAN PRATT, *Department of Zoology, University of Wisconsin*.

THE EFFECT OF VARIOUS ENVIRONMENTAL CONDITIONS ON THE VIABILITY OF THE OVA OF *CAPILLARIA COLUMBAE* (RUD.)

The author has shown that *Capillaria columbae* is pathogenic for chickens (in press, Jour. Parasit.). As a part of the larger study, the resistance of the ova of this parasite to different environmental conditions was investigated.

Non-embryonated ova were obtained from feces freshly passed by heavily parasitized chickens. Embryonated ova were prepared according to the method described by the author (Jour. Parasit., 1936, 22; p. 291). Parasite-free Barred Plymouth Rocks and White Leghorns, two months old, were fed material containing the ova to be tested. The ova were considered viable when *C. columbae* were found in the intestinal mucosa of the birds from 17 to 21 days after dosing. The method of preparing and exposing the ova to natural weathering conditions was the same as that described by the author for the ova of *Ascaridia lineata* (elsewhere in this issue).

Newbigin and Morgan (1936, Scottish Journal of Agriculture 19, p. 162), have noted that poultry runs that had been rested for eight months still harbored viable ova of *Capillaria spp.* Further proof that the ova of *C. columbae* are long lived when exposed to natural weathering is indicated in the following results. Embryonated ova exposed to weathering in the shade survived from Oct. 21, 1935, to Nov. 9, 1936, 384 days. They were dead when tested on March 9, 1937, 500 days. Many of the ova were destroyed during the period of exposure since the number of worms found in the birds dosed with the aged material was much less than that found in the test birds at the beginning of the experiment. It is interesting to note that the embryonated ova of *A. lineata* exposed in an identical manner, did not survive the winter of 1935-36. Embryonated ova of *C. columbae* exposed to weathering in the sun survived from Nov. 2, 1935, to March 27, 1936, 146 days. The ova were non-viable when tested on May 11, 1936, 191 days. In another test, non-embryonated ova were exposed to the sun on June 2, 1936. These ova were found to have embryonated naturally on July 8, 1936. When tested on Oct. 9, 1936, they were found to be non-viable, 129 days after the beginning of the experiment.

When embryonated ova were thoroughly dried by means of an electric fan, they did not survive fourteen days, the shortest period determined.

Embryonated ova in fecal residue (50% moisture) were placed in a freezing compartment of an electric refrigerator at a temperature of -1 degree C. These ova survived continuous freezing for 316 days but not for 346 days. The freshly embryonated ova of *C. columbae* were not destroyed by the 'shocking' effect of a rapid temperature drop to freezing. Freshly embryonated ova of *A. lineata* did not survive this treatment.

Embryonated ova of *C. columbae* in putrefying feces at room temperature survived for 20 days, but not for 28 days. Non-embryonated ova in putrefying feces survived for 42 but not 57 days. Examination of the putrefied material at the end of the latter experiment showed that some of the ova contained degenerated embryos. Apparently a few of the ova had embryonated naturally.

Embryonated ova of *C. columbae* suspended in water were viable after being heated to 40 degrees C. for 5 minutes. Heating suspensions to 50 degrees C. or higher for the same length of time destroyed the ova.

In conclusion it can be stated that embryonated ova of *C. columbae* are more resistant to weathering in the shade and to cold than those of *A. lineata*. However, drying is rapidly fatal to the embryonated ova and is probably the significant lethal factor when they are exposed to the direct rays of the sun. From a practical point of view the data presented indicate that the destruction of ova of *C. columbae* would take place in the shortest time on a well drained unshaded poultry range.—P. P. LEVINE, *Department of Pathology and Bacteriology, New York State Veterinary College, Cornell University, Ithaca, N. Y.*

THE EFFECT OF PHENYLHYDRAZINE HYDROCHLORIDE ON THE PERIPHERAL BLOOD PICTURE IN CANARIES

Phenylhydrazine hydrochloride ($C_6H_5.NH.NH_2.HCl$) is a powerful protoplasmic poison in large doses, causing fatty degeneration of the liver and marked destruction of the red blood cells. The latter effect has been used clinically to reduce the number of erythrocytes in erythremia (*Polycythemia vera*). Its use, however, is attended with some danger.

During a series of experiments on the susceptibility of young red cells to malaria (*Plasmodium cathemerium*) in canaries, phenylhydrazine hydrochloride was used to increase the number of young red cells in the peripheral blood. Because of the marked difference between "young red cells" and "old red cells" in birds, the peripheral blood picture can be studied to great advantage in bird blood. The following results present the changes in the peripheral blood of canaries after four doses of phenylhydrazine hydrochloride, and may be of some value in correlating similar changes in humans undergoing treatment for erythremia.

About fifteen birds have been treated with phenylhydrazine hydrochloride. Results varied with the dose given and the length of time elapsing between doses. The results on three birds given in the accompanying graph serve as typical examples of the blood picture when phenylhydrazine hydrochloride is given in several doses by mouth. 100 mgm. of phenylhydrazine hydrochloride was dissolved in 25 cc. of water, and 250 mgm. of this solution was given to each bird. A fresh solution was used each day. Treatments were given with a 1 cc. tuberculin syringe fitted with a short piece of catheter tubing. The tubing was thrust down the throat of the bird and the dose injected directly into the stomach.

Normal canary blood contains three distinct types of erythrocytes: (1) nucleated basophilic corpuscles with a large, polychromaphilic nucleus, and cytoplasm staining only with basic dyes. These occur in fractions of 1 per cent; (2) nucleated amphophilic corpuscles with an oval nucleus, and cytoplasm staining either with acid or basic dyes. These cells are polychromaphilic and make up from 1 to 6 per cent of the red cells in the peripheral blood; (3) nucleated oxyphilic corpuscles with an oval, homogenous nucleus, showing little or no polychromasia.

Polychromasia is recognized as a sign of immature red cells and marrow stress; therefore, types (1) and (2) are called "young red cells" and type (3) "old red cells." Types (1) and (2) can readily be recognized in blood smears stained with Giemsa stain. The cytoplasm of the younger cells stains gray or blue and the nucleus a mottled purple, with large clumps of deeply stained chromatin. The cytoplasm of old cells stains light orange or takes no stain, and the nucleus stains uniformly throughout.

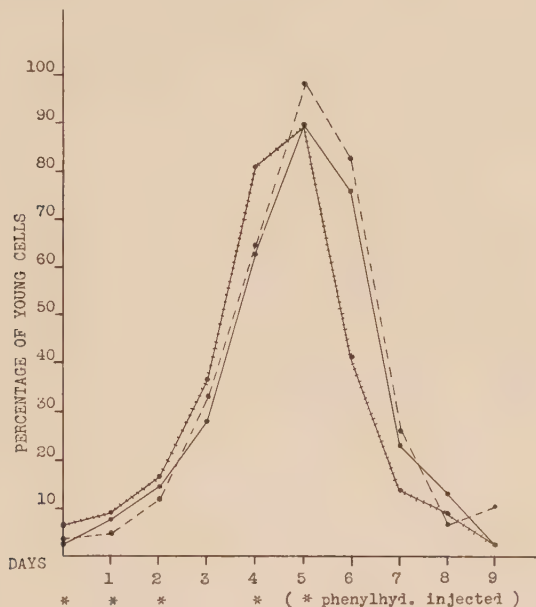


FIGURE 1. Showing the rise in percentage of young red cells in the peripheral blood of three birds after injections of phenylhydrazine hydrochloride as indicated.

The graph shows a marked rise in the young cells very soon after the first injection of phenylhydrazine hydrochloride, reaching a peak on the fifth day after the first injection. On the seventh and eighth days (the last injection being on the fourth day) many of the cells were of intermediate types and clear-cut distinctions could not be made between old and young cells. Those cells, however, in which the nucleus showed little polychromasia and was distinctly smaller than those of other cells were tabulated as old cells. Percentages were based on an average count of five hundred red cells.

I am indebted to Dr. Robert Hegner for helpful suggestions and criticism, to Walter Landsberg for suggesting the use of phenylhydrazine hydrochloride, and to Miss Lydia Eskridge for determining its minimum lethal dose for canaries. REGINAL HEWITT, *Department of Protozoology, School of Hygiene and Public Health, The Johns Hopkins University.*

ON THE LIFE CYCLE OF *CONTRACAECUM SPICULIGERUM* (Rud.)

Observations made on the food of the common cormorant, *Phalacrocorax auritus auritus* (Lesson) in October, 1935, at Rock Falls, Illinois, while on the Illinois Natural History Survey's laboratory boat on the Rock River, disclosed that their chief diet was young gizzard shad, *Dorosoma cepedianum* (Le Sueur). Two adult birds examined had each over 200 nematodes of the genus *Contracaecum* in the proventriculus. Two of numerous gizzard shad examined had im-

mature *Contracaecum* encysted in their mesenteries. The smallest worms from the cormorants were the same as those from the mesenteries of the shad.

On October 30, 1936, two more cormorants from the Chautauqua Lake District, Havana, Illinois, were secured through the courtesy of Dr. David H. Thompson of the Illinois Natural History Survey. Approximately 200 worms were taken from the proventriculus of one bird, with their bodies nearly buried in the ducts of that organ. Eggs with the average measurement of $58\mu \times 63\mu$ were dissected out of the uterus of six female worms, measuring 2 to 2.5 cm. in length, and placed after washing in normal saline and physiological saline solution in distilled water. Just enough water was used to cover them in Petri dishes. At this time most of the eggs were in the morula stage. Observed again on November 1, 1936, the tadpole stage was prevalent. Three days later larvae were turning about in the eggs with the first molt and cuticular tooth present. A second molt within the egg was observed the morning of the following day. By afternoon most of them had hatched. They swam rapidly about and became entangled in clumps in the bottom of the dishes. The loose anterior ends of the cuticular sheaths seemed adhesive, as many attached directly by these anterior ends to the bottom of the dish and moved freely in their sheaths, coiling and waving to and fro. When detached they swam vigorously but did not respond readily to heat on a slide as will hookworm larvae. There was little or no response to light stimulus. Measurements of larvae including the two sheaths at this time showed a total length of 528μ . The larvae within the sheath measured 368μ in length. Observations made on November 11 showed that a third molt was in progress with a cuticular tooth still present. Eighty per cent of the larvae were alive and active on November 11, and by November 26 only three or four moved feebly in their attached positions in the Petri dishes. Six large females were placed in distilled water in a Petri dish and left in the ice box until December 7, 1936. Their eggs were dissected out and treated as before. They hatched 100 per cent December 12, 1936. Several adult worms in a Petri dish were placed in the ice box October 31, 1936. The distilled water which covered them evaporated so that they stuck to the bottom of the dish. When examined March 16, 1937, the eggs were fully embryonated and hatched almost as soon as they were dissected out of the uteri of the worms.

Two more cormorants were obtained; one had a natural infection of 50 female and 60 male *Contracaecum*, the other had 61 males and 57 females. The adult females were placed in the ice box for future use.

Infection experiments were begun at 10:00 A. M., November 11, 1936. Five guppies, *Labistes reticulatus*, 20 mm. long, were placed in a two inch stender dish with several hundred infective larvae. One fish examined at 10:00 A. M. the following day had 15 *Contracaecum* larvae moving about in the intestine. The first two molts had been shed. On November 14, a Petri dish full of adhering worms was placed on the bottom of a $6'' \times 10'' \times 8''$ aquarium with 25 guppies. Two guppies, 8 mm. long were found dead November 16, and examined. One had three worms in the intestine with the first two molts shed and one worm in the body cavity which measured 388μ in length. The other fish had 155 similar larvae in the intestine.

A five inch black bass, *Aplites salmoides* (Lacepede), obtained from the Natural History Survey, was fed 10 parasitized guppies. All but two were eaten the same day, November 18. These two fish were found dead November 19. One of these individuals 3 cm. in length when examined had seven worms in the intestine with the first two molts shed and the other, a 2 cm. long fish, had seven similar worms in the intestine; these worms averaged 353μ in length. The bass was examined November 21 and one larvae was found encysted in the mesenteries. It measured 1.117 mm. in length and still possessed a cuticular tooth. This experiment was repeated with a 5 inch black bass December 16, but no encysted larvae were found December 29, when it was examined.

Direct infection of six day-old chicks was tried January 21, 1937, and each given about 200 larvae, but when examined after three hours dead larvae were found in the rectum and feces. Five guppies that had been infected for one week were fed to each of five day-old white leghorn chicks, January 22, 1937, but no larvae were found when examined January 30, with the same results.

A series of similar experiments was carried out with adult white pekin ducks which were negative; both experimental and natural infections of worms were fed them. It was evident that these birds were not adapted to *Contracaecum*. A guppy examined February 12, 1937, from a lot infected November 13, 1936, had one larval worm encysted in the mesentery near the liver, walled in by host tissue; it measured 1.345 mm. in length. The oesophageal diverticula, two glands, measured 151 μ in length; their content was granular and was seen to move by two channels into ducts in the oesophagus. Two more guppies from this same lot examined February 19, 1937, had each 3 large larvae encysted in the mesenteries near the liver and one had a worm encysted on the liver capsule. All the worms still retained the cuticular tooth and three lips were evident under this sheath. The smallest worms obtained from the cormorants were similar. Natural infections of larvae have been obtained from fish taken at Havana, Illinois: in the warmouth bass, *Chaenobryttus gulosus* (Cuv. and Val.); bluegills, *Helioperca macrochira* (Raf.); and black bass. After two molts in the egg, *Contracaecum* larvae hatch and are infective to fish that eat them. Transfer of infective larvae from minnows to carnivorous fish is possible. Cormorants become infected by eating the worms encysted in fish.—LYELL J. THOMAS, *Department of Zoology, University of Illinois, Urbana, Illinois.*

ON THE SECOND INTERMEDIATE HOST OF *LOXOGENES LIBERUM* SENO, 1907

During my parasitological survey of Lake Kobata I found some nymphs of *Crocothemis servilia* Drury infected in the abdominal cavity with trematode cysts, which were ascertained by feeding experiment to contain metacercariae of *Loxogenes liberum* Seno, 1907. The wall of the cyst consists of three layers, of which the outer is a lamellar connective tissue membrane, the middle a thick gelatinous one containing some granular substance, and the inner a thin transparent one, measuring 0.22–0.25 mm. in diameter. As liberated from the cyst and measured under a cover glass the metacercaria covered with minute spines is about 0.28 mm. long by 0.25 mm. broad; the oral sucker and pharynx are 60 μ and 19 μ in

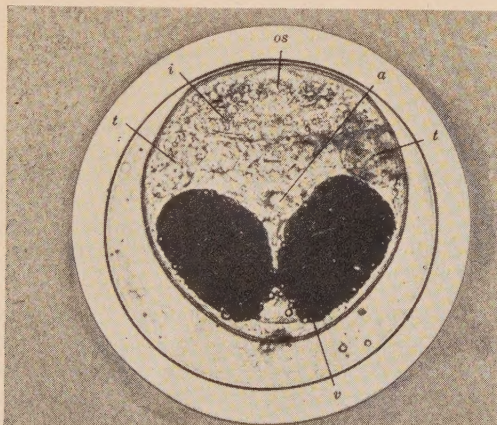


FIG. 1. Cyst of *Loxogenes liberum* from nymph of *Crocothemis servilia*; $\times 75$.

diameter respectively. The esophagus is very short, and the widely divergent ceca extend each to the anterior end of the testis. The acetabulum, 56μ in diameter, is situated immediately behind the middle of the body. The male genitalia, especially the testes and cirrus pouch, are well developed; the oval testes, 67μ long by 42μ broad, lie symmetrically near the lateral margins of the body on a level with the anterior end of the acetabulum. The anlagen of the female genitalia except the ovary are unable to trace with certainty. The V-shaped excretory vesicle with a ventroterminal opening is very conspicuous and distended with fine granular concretions; each arm reaches to the posterior end of the testis of its own side, where it gives off at the tip a collecting vessel running forward and bifurcating into an anterior and a posterior tubule near the intestinal termination.

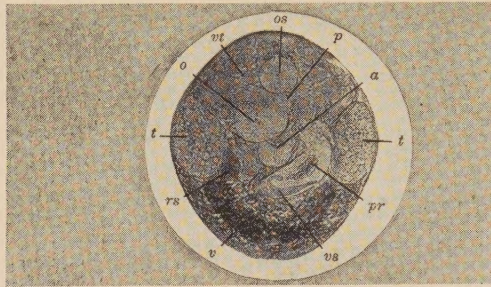


FIG. 2. Experimentally obtained adult of *Loxogenes liberum*; $\times 35$.

Abbreviations used in Figures

a acetabulum, *i* intestine, *o* ovary, *os* oral sucker, *p* pharynx, *pr* pars prostatica, *rs* receptaculum seminis, *t* testis, *v* excretory vesicle, *vs* vesicula seminalis.

On May 28, 1936, five cysts were fed to an adult *Rana nigromaculata* taken from the locality, where *Loxogenes liberum* is not known to occur. Ten days later the frog was killed and three mature worms were recovered. In one of these the uterus had formed definite preovarian loops, and the eggs had been embryonated. They were elliptical, thin-shelled, and measured $32\text{--}36\mu$ long by $14\text{--}17\mu$ broad.—SATYŪ YAMAGUTI, *Laboratory of Parasitology, Kyoto Imperial University, Kyoto, Japan*.

A NEW SPECIES OF TETRAONCHINAE FROM *LEPOMIS SYMMETRICUS*

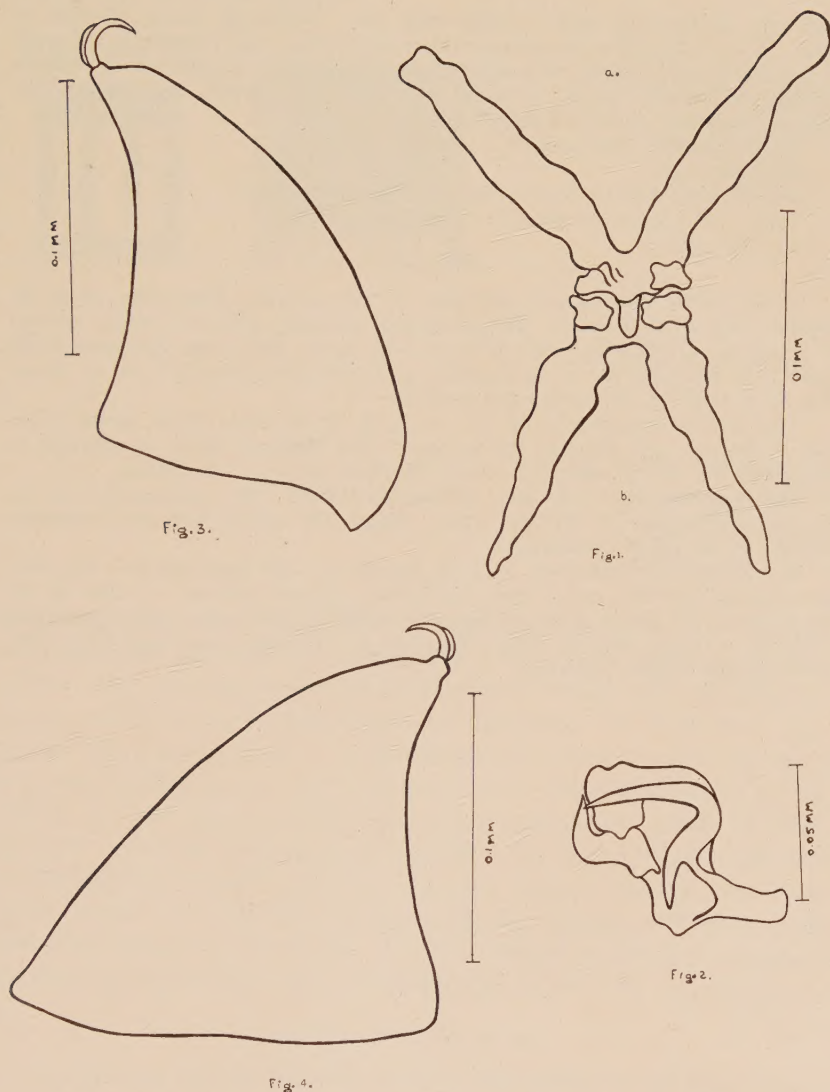
During the course of investigation on gyrodactyloid trematodes occurring on the gills of the Centrarchidae of Louisiana, a large number of monogenetic trematodes have been encountered all of which fall into the sub-family Tetraonchinae.

The host specimens used in the work underway at the present time were all collected from the fresh-water lakes and bayous near Baton Rouge, Louisiana.

The freezing method used in obtaining the parasites from the gills was similar to that used by (Mizelle 1936).

Actinocleidus triangularis sp. nov. (Figs. 1a, 1b, 2, 3, 4)

Tetraonchinae; worms of relatively large size, robust or stout. Length 1.09 mm. (0.88–1.26 mm.), width 0.2 mm. (0.176–0.2 mm.) at level of pharynx. Haptor situated at right angles to body, discoidal. Peduncle terminates in center of haptor. Haptor length 0.293 mm. (0.272–0.30 mm.), width 0.373 mm. (0.352–0.400 mm.). Width of peduncle at junction with haptor 0.192 mm. (0.176–0.208 mm.). Anchors triangular in shape, basal portion large, points of anchors on



same side of haptor oppose one another. Length of ventrally situated anchor (Figure 3) 0.192 mm. (0.176–0.208 mm.), width of base 0.121 mm. (0.112–0.144 mm.). Length of dorsally situated anchors (Figure 4) 0.186 mm. (0.176–0.208 mm.), width of base 0.124 mm. (0.118–0.128 mm.). Bars articulated, V- shaped. Distance between points of dorsal bars (Figure 1, a) 0.112 mm., length of arm of V, 0.138 mm. (0.128–0.144 mm.). Distance between points of ventral bars (Figure 1, b) 0.096 mm., length of arm of V, 0.096 mm. Hooks fourteen in number, situated around the margin of the haptor, each hook differentiated into base, shaft, sickle-shaped termination and opposable piece. Bases slightly shorter than shafts. Head region contains three pairs of head organs located around the anterior margin of the head. Four eyespots. Pharynx diameter 0.110 mm. (0.108–0.111 mm.). Intestine confluent posteriorly. Gonads located in the middle third of the body. Testis above and slightly posterior to ovary. Testis length

0.148 mm. (0.144–0.151 mm.), width 0.078 mm. (0.072–0.09 mm.), elliptical in shape. Cirrus articulated to accessory piece at proximal end (Figure 2). Length of cirrus 0.03 mm., length of accessory piece 0.025 mm. Vagina on left margin of body, midway the length. Vaginal tube short and straight, length 0.025 mm. Seminal receptacle dumb-bell shaped, large.

Host—*Lepomis symmetricus*

Location—Gills

Distribution—United States (Baton Rouge, Louisiana)

Types and cotypes—in author's collection

Discussion

Actinocleidus triangularis conforms to the generic description given by Mueller, 1937, in all respects. The cirrus and accessory piece are similar to that of *A. articularis* (Mizelle, 1936) in every way except size, these structures being larger in *A. triangularis*. *A. triangularis* is further differentiated from *A. articularis* by its larger body, haptor and anchors.

This species is strikingly different from all the members of the genus in regard to the size and shape of the anchors. This character alone is sufficient to distinguish it from all other previously described species of the genus.

Due to an oversight in a recent publication Mueller, 1937, designated a species as *Actinocleidus incisor* (Mizelle, 1934), though the species had been originally ascribed to the genus *Cleidodiscus*.

In a recent correspondence with Dr. Mueller, he has suggested that the present writer correct this error, since *Cleidodiscus incisor* does not conform to the characters of the genus *Actinocleidus* and should be left in its original genus as *Cleidodiscus incisor* Mizelle, 1936.—WILLIAM A. SUMMERS, Louisiana State University, Baton Rouge, Louisiana.